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DIAGNOSIS AND PROGNOSIS OF COAGULOPATHIES IN HORSES WITH COLIC

Memòria presentada per

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per optar al Grau de

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Director de la Tesi

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Eduard José Cunilleras, LV, PhD, Dip ACVIM, professor lector del Departament de Medicina i Cirurgia Animals de la Facultat de Veterinària de la Universitat Autònoma de Barcelona,

CERTIFICO:

Que la Tesi que porta el títol: “DIAGNOSIS AND PROGNOSIS OF COAGULOPATHIES IN HORSES WITH COLIC”, de la que n’és autora la Llicenciada en Veterinària **Carlota Cesarini Latorre**, s’ha realitzat a la Facultat de Veterinària de la Universitat Autònoma de Barcelona, sota la meva direcció.

I per a que així consti, a efectes de ser presentada com a Tesi Doctoral per optar al títol de Doctor en Veterinària, signo aquest certificat a Bellaterra, 20 de setembre de 2013.

Eduard José Cunilleras

“Siempre llevaba una chaqueta blanca,

parecía muy oficial y eficiente, y,

en un costado de la camioneta,

con letras enormes (…) se leía:

“Arthur Lumley, M.C.P. Especialista en Caninos y Felinos”.

(…) Sin embargo, aquellas letras “M.C.P.” no me resultaban familiares

y él se mostró algo misterioso cuando se lo pregunté.

Por fin conseguí averiguar lo que significaban:

“Miembro del Club de Perros”.”

James Herriot, en *Todas las Cosas Brillantes y Hermosas*

Va per tu, Lluís

Para ti, mamá

Agraïments

De entrada, i principalment, donar les gràcies al Lluís. Les que ja li vaig donar i totes les que li dec, gràcies infinites, GRÀCIES en majúscula. Per escoltar-me sense presses sempre que vaig necessitar parlar, des que tenia 20 anys i no sabia què fer amb la meva vida, però havia de ser amb cavalls, fins que en tenia 33 i, en part gràcies a tu, ja portava més de 10 anys treballant amb ells; per valorar-me més que jo a mi mateixa; per les “bronques”; per les presses; per no deixar de tirar del carro....Et trobo a faltar però sempre estaràs amb mi. Gràcies especials Luiggi, perquè aquesta tesi va ser idea teva i tu ets la seva ànima.

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Index

Introduction and

Review of the Literature

Health and Disease of the Hemostatic System

Overview of the Healthy Hemostatic System

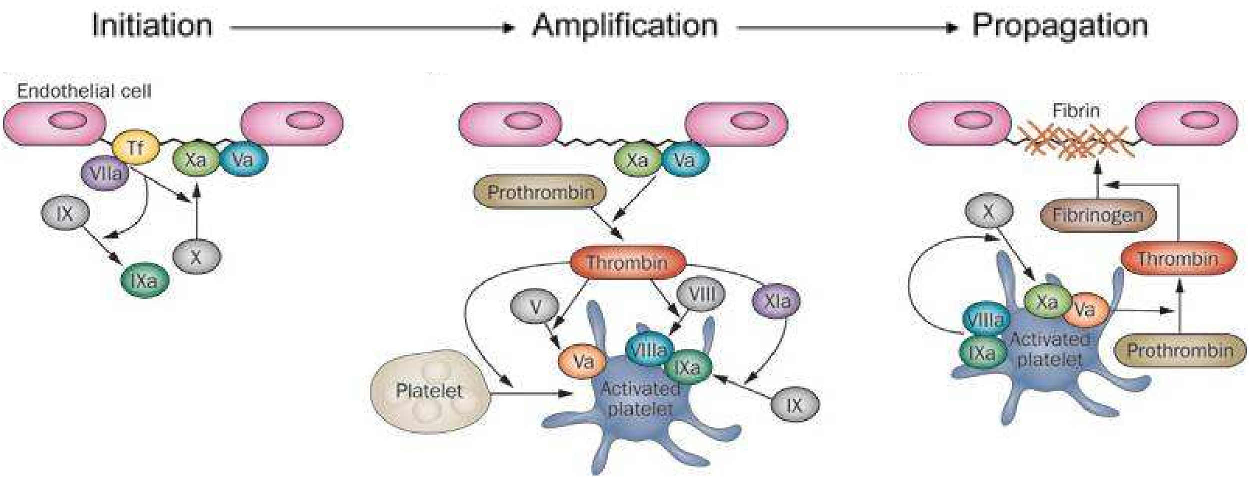
The main objective of the hemostatic system is to prevent hemorrhage while maintaining adequate blood flow to tissues. In order to accomplish this goal, the first step is an efficient clot formation and retraction, but equally important is the corresponding fibrinolysis that follows. As vascular defects heal, the fibrinolytic plasma protein, plasmin, slowly degrades cross-linked fibrin to prevent excessive intravascular or extravascular fibrin deposition and re-establish blood flow, restoring appropriate end-organ perfusion. This balance between clot formation and lysis is very well regulated and receives significant contributions from inflammatory cells and cytokines (*Dallap 2004, Brooks 2008*).

The complex process of hemostasis involves four major components: blood vessels, platelets, the coagulation cascade, and regulators of coagulation (*Lassen 1995*). The original model of coagulation involves the "intrinsic" and "extrinsic pathways", two different series of activation reactions that converge in a "common pathway" to produce thrombin, which transforms plasma fibrinogen into an insoluble fibrin clot. Although this "cascade" model helps to understand in vitro coagulation, an alternative "cell-based" model has been recently developed, which reflects better the in vivo formation of a hemostatic plug (*Roberts 2006*).

The **cell-based model of hemostasis** consists of three overlapping phases: initiation, amplification, and propagation (**Figure 1**). Each of the three phases of coagulation occurs on a different cell surface. The first "initiation phase" takes place on cells expressing tissue factor (TF) (i.e. fibroblasts). In this phase, the

TF/factor VII complex (TF/FVIIa) transforms factors IX and X to their active forms (FIXa and FXa, respectively), which generate trace amounts of thrombin (FIIa). The second, or "amplification phase", occurs on the platelet as it undergoes activation. In this phase, thrombin mediates the activation of platelets and the coagulation cofactors (factors VIII and V). Finally, the third is the "propagation phase", which happens on the surface of activated platelets and consists on the binding of coagulation complexes (FIXa/FVIIIa and FXa/FVa) to generate large amounts of thrombin that produce polymerized fibrin from fibrinogen (*Brooks 2008*).

Figure 1. Cell-based hemostatic model.



Modified from: *Wisler JW, Becker RC. Oral factor Xa inhibitors for the long-term management of ACS. Nature Reviews Cardiology 2012; 9: 392-401.*

Assessment of Hemostatic Function

Tests used to evaluate the hemostatic function can be classified as tests of primary hemostasis (platelet plug formation) or secondary hemostasis (fibrin clot formation) (*Brooks 2008*). In horses, the most commonly used tests include: a) tests evaluating platelet number and function (platelet counts, bleeding time), b) tests of the coagulation cascade (clotting times, test of anticoagulant proteins); and c) tests of fibrinolytic activity (fibrin degradation products, D-dimers) (*Lassen 1995*).

Prothrombin time (PT) and **activated partial thromboplastin time** (aPTT) are the most useful and practical of the clotting times, because they assess the intrinsic and extrinsic pathways separately. Prolongation of PT suggests a problem in the extrinsic and/or common pathways whereas prolonged aPTT indicates factor deficiencies in the intrinsic and common pathways. If both PT and aPTT are prolonged, that suggests a problem in the common pathway or, what is more common in the horse, a multiple factor problem (*Lassen 1995, Brooks 2008*).

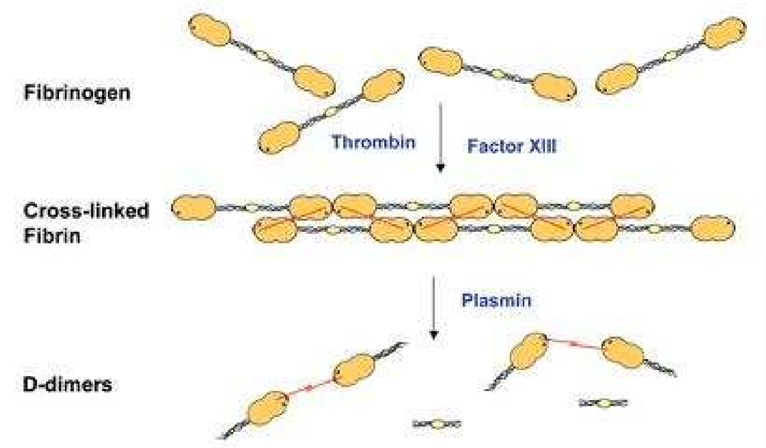
Antithrombin III (AT) is the major anticoagulant protein and the most frequently assayed in the horse. It’s consumed while inhibiting thrombin and several other factors of the coagulation cascade. In thrombotic diseases such as disseminated intravascular coagulation (DIC), increased quantities of AT are consumed in an attempt to control clotting, and its plasmatic concentration decreases (*Lassen 1995*).

Plasmin degrades fibrin (or fibrinogen), to produce a series of progressive cleavage products referred to as **fibrin degradation products** (FDPs). **D-dimer** is the smallest terminal degradation fragment, and consists of two cross-linked D domains of fibrin (**Figure 2**), and its presence is an indicator of fibrin degradation rather than fibrinogen. Quantitative determinations of FDPs and D-dimer are the tests of fibrinolytic activity more readily applicable for clinical use. Assessment of fibrinolytic activity is used, for example, in detecting DIC. When excessive clotting occurs, extremely high levels of fibrinolysis follow, and this can be detected by measuring plasmatic FDPs or D-dimer concentrations (*Lassen 1995*).

Usefulness of D-dimer Quantification

D-dimer is a specific product of the lysis of cross-linked fibrin, and increased plasmatic concentrations are indicative of excessive fibrin formation and degradation within the vascular system (*Monreal 2003*).

Figure 2. Biochemistry of D-dimer formation.



Fibrinogen is converted to cross-linked fibrin polymers through the activity of thrombin and factor XIII. Cross-linked fibrin is degraded by plasmin to generate degradation products containing D-dimer.

From: *Shiu-Ki RH et al. D-Dimer: A Non-invasive Triage Test for Patients with Suspected DVT. Clinical Laboratory News April 2009: Vol 35, 4)*

To have a specific marker for degradation of fibrin, as opposed to fibrinogen, is particularly important in the horse. Given the prevalence of hyperfibrinogenemia in equine patients with a severe inflammatory response, the value of measuring FDPs as an indicator of increased fibrinolysis or hemostatic dysfunction has been questioned (*Dallap 2004*). That's the reason why in most diagnostic laboratories D-dimer has replaced FDP testing for detecting fibrinolysis in DIC (*Stokol 2010*).

D-dimer analysis uses a monoclonal antibody, thereby specifically identifying fibrin degradation products. Semi-quantitative and quantitative latex agglutination kits for D-dimer measurement are commercially available (*Dallap 2004*). In most assay systems, the plasma concentration of equine D-dimer is higher than that of human beings and companion animals, with typical values for healthy horses ranging from 250 to 1000 ng/mL (*Stokol 2005*).

In people, plasma D-dimer concentration has been reported to increase in different physiological and pathological hypercoagulable states, such as exercise or severe systemic disease (neoplasia, sepsis or trauma). In human medicine, D-dimer is considered a very sensitive indicator of DIC, venous thrombosis and pulmonary embolism, and is currently used to identify patients at risk for DIC and their response to antithrombotic therapy (*Bick 1994*, *Monreal 2003*).

In veterinary medicine, increased plasmatic D-dimers have been reported in dogs with DIC (*Stokol 2000, Machida 2010*), thromboembolism (*Nelson 2003, Stokol 2003*), cutaneous vasculitis (*Rosser 2009*) and cancer (*Andreansen 2012*). In horses, an increase in D-dimer concentration has been reported in adults with gastrointestinal disorders (*Dallap 2003, Feige 2003a, Armengou 2005, Stokol 2005, Delgado 2009, Watts 2011*), jugular vein thrombosis and DIC (*Feige 2003a*) and in septic foals (*Armengou 2008*). D-dimer analysis has been used as a prognostic indicator for horses with colic (*Sandholm 1995*) and may indicate a risk factor for development of coagulopathy (*Sellon 2010*). Some authors consider that in veterinary medicine plasma D-dimer concentration should be one of the main criteria in the laboratory diagnosis of DIC and thromboembolic disease (*Monreal 2003*).

Coagulopathy, the Hemostatic Dysfunction

From a clinical perspective, hemostatic disorders or coagulopathies can be grouped according to two main pathophysiological processes: excessive activation (hypercoagulable states) or deficient activation (hypocoagulable states) of the coagulation system (*Monreal 2008&2009*).

Systemic activation of platelets and clotting factors, or lack of regulatory proteins, produces a thrombotic tendency. The **hypercoagulable states** most commonly diagnosed in hospitalized horses include jugular thrombophlebitis, DIC and other more sporadic thrombotic events, such as arterial thrombosis, pulmonary thromboembolism or congenital deficiency in anticoagulant proteins (*Jones 1987, Morris 1989, Edens 1993, Traub-Dargatz 1994, Triplett 1996, Brianceau 2001*).

On the other hand, deficiencies in the amount or function of platelets and clotting factors may result in clinical signs of hemorrhage. **Hypocoagulable states** are less frequently observed in hospitalized horses than hypercoagulable states, but they include thrombocytopenia, vitamin K deficiency, severe liver failure, rare inherited hemostatic disorders in young animals (like Hemophilia A, von Willebrand’s disease, prekallikrein deficiency, Glanzmann-thrombasthenia) and situations of excessive consumption of coagulation factors, such as profuse hemorrhage or the bleeding form of DIC (*Geor 1990, Brooks M 1991, Fry 2005, Monreal 2008*).

Cross-talking between Inflammation and Hypercoagulation

Coagulopathic dysfunction is no longer considered a separate primary disease process, but is viewed instead as part of a massive and imbalanced systemic inflammatory response (*Dallap 2004*). Inflammation initiates coagulation via tissue injury, induces TF expression, and platelet and leukocyte activation and microvesiculation. Fibrinolysis is also stimulated by inflammation. Coagulation, in turn, stimulates inflammation, because active coagulation factors have proinflammatory properties. Consequently, a self-perpetuating cycle develops (*Stokol 2010*).

The severity of the inflammatory response to an inciting event may be extremely critical in the development of coagulopathy and may influence patient outcome (*Marshall 2001*). The amplification of the host response, from simple inflammatory process to sustained coagulopathy, could result from alterations in endothelial cell function secondary to stimulation by pro-inflammatory cytokines, like tumor necrosis factor and interleukins 1 and 6, in combination with the contribution of activated complement components (*Taylor 1994*).

To date, it is still unclear if the main cause of the end-organ damage associated to certain coagulopathies is the hypoxemia and tissue ischemia resulting from the microvascular fibrin accumulation or the excessive up-regulation of the inflammatory response (*Dallap 2004*).

Disseminated intravascular coagulation (DIC)

What is DIC?

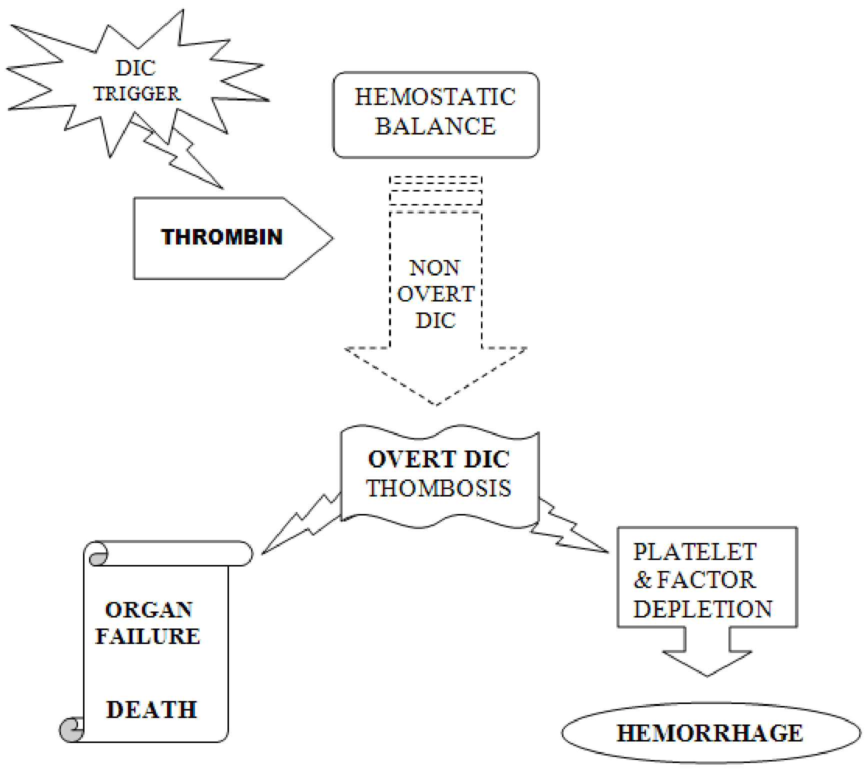
Historically, DIC was initially described as a hemorrhagic diathesis resulting from intravascular consumptive coagulation (*Rodriguez-Erdman 1965*). Later on, DIC was considered as part of the various recognized stages of hemostatic dysfunction: hypercoagulability, compensated or subclinical DIC, and fulminant or uncompensated DIC (*Dallap 2004*).

Currently, the International Society of Thrombosis and Hemostasis (ISTH) defines DIC as a syndrome characterized by the systemic activation of blood coagulation, which generates intravascular fibrin, leading to thrombosis of small and medium-sized vessels, and eventually organ dysfunction” (*Taylor 2001*).

Disseminated intravascular coagulation is not a primary disorder, but a syndrome that always develops secondary to an underlying disease, which induces systemic activation of coagulation and simultaneously depresses anticoagulant mechanisms (*Stokol 2010*). Regardless of the inciting disease, the DIC process results in systemic small vessel thrombosis (*Brooks 2008*). Many different primary disorders trigger DIC, and its clinical and laboratory manifestations vary among patients and change over time, complicating its recognition and treatment. Endotoxemia (secondary to gastrointestinal disorders) and sepsis are the main causes of DIC in adult horses and neonatal foals, respectively (*Dolente 2002*, *Stokol 2005, Armengou 2008*).

The **pathogenesis of DIC** is complex (**Figure 3**). In some cases (i.e. sepsis) the “trigger” is thought to be the induced expression of TF in the vascular space (*Brooks 2008*). In a positive feedback cycle, trace amounts of thrombin are amplified by intrinsic pathway reactions to produce massive amounts of thrombin (*Hoffman 2001, Toh 2005*). This thrombin burst splits soluble fibrinogen into fibrin polymers, to form the final cross-linked fibrin clot (*Stokol 2010*).

Figure 3. Overview of DIC.



Adapted from*: Brooks MB. Equine coagulopathies. Vet Clin North Am Equine Pract 2008;24:335-355.*

The development of DIC depends on one hand on the location and severity of the inciting stimulus, but also on the ability of natural inhibitors to regulate the procoagulant hemostatic response (*Stokol 2010*). The term **"non-overt DIC"** describes a phase of systemically activated coagulation still compensated by natural anticoagulants. Patients with non-overt DIC are considered hypercoagulable and they are at risk for widespread microvascular fibrin deposition. As the underlying disease progresses and natural coagulation inhibitors become overwhelmed, TF expression and thrombin generation persists. **“Overt DIC”** refers to an active thrombotic syndrome, with fibrin deposition throughout multiple organs (i.e. heart, lungs, liver, kidney, central nervous system) (*Stokol 2010*).

Consequences of DIC

The systemic uncontrolled generation of thrombin produces **diffuse microvascular fibrin thrombi**, characteristic of overt DIC. Widespread microvascular thrombosis decreases blood flow to vital tissues thereby causing hypoxic injury, cell death, and organ failure; factors ultimately responsible for the high morbidity and mortality of DIC (*Bateman 1999, Bick 2002, Dolente 2002*, *Toh 2005*). The syndrome has often been described in horses with clinical conditions associated with poor prognosis (*Dolente 2002, Dallap 2003, Cotovio 2007a*). Furthermore, horses in DIC have been reported to be eight times more likely to die (*Dolente 2002*).

As it has been developed in the previous section, in cases of DIC associated to septic or non-septic inflammation, a self-perpetuating cycle develops between the **systemic inflammatory response** and the hemostatic activation, which further complicates the clinical situation and worsens patient outcome.

Hemorrhagic DIC as a result of consumption coagulopathy develops frequently in dogs, but is rare in horses. If present, excessive bleeding is usually due to thrombocytopenia resulting in petechial and ecchymotic hemorrhages (*Lassen 1995*).

Every animal with a triggering primary disease should be considered at risk for developing DIC and screening tests should be performed to detect overt DIC (*Stokol 2010*). Confirmation of DIC is challenging because animals may be examined at any point of the syndrome continuum, clinical signs may be subtle or nonspecific, and individual laboratory tests are variably sensitive and specific for this syndrome (*Stokol 2010*). Despite having low diagnostic utility, histopathologic evidence of fibrin thrombi throughout the microvasculature in biopsy or necropsy specimens is considered a "gold standard" test for DIC in veterinary medicine (*Stokol 2010*). There is no pathognomonic sign or single laboratory finding indicative of DIC. Diagnosis is based on the presence of an initiating disease and a combination of clinical and laboratory abnormalities (*Bick 1994, Kirby 2000, Stokol 2005*). **Laboratory abnormalities associated with DIC** include evidence of (1) procoagulant activation, (2) fibrinolytic activation, (3) inhibitor consumption, and (4) biochemical evidence of end-organ failure'' (*Bick 1994*). Traditional criteria for diagnosis of overt DIC in animals include a combination of two or more test abnormalities (thrombocytopenia, prolonged coagulation times, hypofibrinogenemia, low AT, and high FDPs or D-dimer concentration) (*Thomas 1998, Stokol 1999, Dolente 2002, de Laforcade 2003, Stokol 2005, Estrin 2006, Armengou 2008*). The apparent diagnostic utility of these tests varies depending on species and stage at presentation. In the equine veterinary literature, patients are considered to have subclinical DIC if they have abnormalities in 3 of 6 traditionally available tests (platelet count, PT, aPTT, AT, fibrinogen, and either FDPs or D-dimers) (*Welch 1992, Dallap 2009*). Mild to moderate thrombocytopenia is not a consistent finding in horses with overt DIC (0-64% sensitivity) (*Dolente 2002, Stokol 2005*). The aPTT appears to be more sensitive for detecting DIC in animals than the other routine coagulation screening tests (*Scott-Moncrieff 2001, Dolente 2002, Stokol 2005, Estrin 2006*). Low AT activity is one of the more sensitive tests for diagnosis of DIC in horses (89-93% sensitivity) (*Dolente 2002, Stokol 2005*). Fibrinogen concentration is rarely decreased in horses and is not considered a reliable parameter for diagnosis of DIC (*Johnstone 1986a, Dolente 2002*). D-dimer seems to be less sensitive for DIC in horses (50% sensitivity) than in dogs (*Stokol 2005*).

In human medicine, a practical **scoring system for consistent diagnosis of overt DIC** has been developed by the ISTH, including results of four readily available laboratory tests: platelet count, PT, fibrinogen, and fibrin-related markers (soluble fibrin, FDP or D-dimer) (*Taylor 2001*). The scoring system is applied to patients at risk of DIC and it has shown a correlation with outcome (*Wada 2013*). Nevertheless it is still undergoing validation to further refine its diagnostic utility.

Diagnosing non-overt DIC is even more challenging. Routine coagulation screening tests are considered insensitive due to the fact that they should detect coagulation factor deficiencies rather than accelerated coagulation. For that reason a second **scoring system for the diagnosis of non-overt DIC** has been developed by the ISTH. It is also based on routine laboratory tests but takes into account the underlying disease and it requires serial testing to evaluate the dynamic nature of non -overt DIC (*Taylor 2001, Wada 2013*). This algorithm has been modified and applied to critically ill dogs (*Wiinberg 2006*). Currently, scoring systems developed to define DIC are not widely used in veterinary medicine but nevertheless, due to species-differences in underlying diseases and diagnostic test sensitivity for DIC, it is unlikely that a single scoring system will apply across species (*Dallap 2004, Stokol 2010*).

The need for better strategies to diagnose early DIC is recognized both in human and veterinary medicine. Early diagnosis will hopefully improve clinical outcome through the use of earlier and more directed therapeutic interventions (*Bateman 1999, Donahue 2005, Wiinberg 2008*).

Managing DIC

The best chance for successfully treating a coagulopathy in the equine patient is early identification, removal or treatment of the inciting cause, and therapy directed at restoration of normal physiologic hemostasis (*Dallap 2004*).

Treatment of the underlying disease is considered paramount in current recommendations for DIC management in human medicine, and in many cases, once the underlying disorder is properly managed, DIC will spontaneously resolve (*Wada 2013*). General supportive treatments in horses may include ensuring adequate perfusion (i.e. fluid therapy) and controlling endotoxemia (i.e. flunixin meglumine, polymixin B). However, some cases require additional treatment, and close monitoring of any abnormal hemostatic parameters and/or any overt clinical signs should be used to direct therapy toward the most affected area of the coagulation system (*Dallap 2004*). Currently available options for treatment of coagulopathies in the equine patient include administration of fresh frozen plasma, platelet-rich plasma, heparin (unfractionated or low-molecular weight), or aspirin (*Dallap 2009*).

In horses, good quality **fresh-frozen plasma** may provide coagulation factors and variable amounts of AT (if properly stored and handled) and **platelet-**

rich plasma can be obtained relatively easily in an equine referral hospital by using platelet collection kits and two-speed centrifugation techniques (*Dallap 2004*). Administration of platelet concentrate and fresh frozen plasma **(**blood component therapy) should not be instituted on the basis of laboratory results alone, but reserved for those patients with active bleeding or requiring an invasive procedure (high risk for bleeding complications) (*Wada 2013*).

Heparin is the most common anticoagulant used in the horse. At low doses, heparin increases the activity of AT, inactivates factor Xa, and inhibits the conversion of prothrombin to thrombin. At higher doses, heparin inactivates thrombin, preventing conversion of fibrinogen to fibrin (*Dallap 2004*). **Low molecular weight heparins** (LMWH), such as dalteparin or enoxaparin, have been reported to have less adverse effects than unfractioned heparin (*Feige 2003b*) and they are usually preferred for treatment of equine patients if there are no financial constraints. However, controlled prospective clinical trials to prove efficacy of heparin in reducing incidence of coagulopaties or improving patient outcome in horses are currently lacking.

Acetylsalycilic acid (aspirin) is commonly used as an inexpensive antithrombotic drug in equine veterinary medicine, despite no prospective studies have demonstrated such an effect in the horse (*Dallap 2009*). Aspirin irreversibly inhibits platelet cyclooxygenase, preventing the metabolism of arachidonic acid (AA) to thromboxane A2. Nevertheless, equine platelets undergo reversible aggregation when exposed to AA (*Meyers 1979*) and experimental studies report a lack of aggregation response when they are exposed to AA or thromboxane analogues (*Meyers 1979*, *Heath 1994*, *Pelagalli 2002*, *Segura 2005*). Furthermore, more recent studies found that the effects of aspirin on equine platelets do not appear to be reliable (*Brainard 2011*). In conclusion, literature suggests that cyclooxygenase inhibitors may have limited efficacy in preventing thrombosis in horses (*Heath 1994, Segura 2006*).

Successful results have been reported in human medicine with other anticoagulant therapies, like recombinant human activated Protein C, AT, and factor VII administration. Nevertheless, prospective clinical studies in the horse have not been done and financial constraints may limit the use of such therapies in equine medicine (*Dallap 2004*).

Coagulopathies in equine gastrointestinal disorders

What we know

A systemic coagulopathy consistent with a marked **hypercoagulable state** can be frequently found in horses with severe colic (*Monreal 2000, Dallap 2004, Armengou 2005, Smith 2005, Delgado 2009*). Gastrointestinal diseases, especially those that result in increased circulating endotoxin concentrations (i.e. ischemic and severe inflammatory disorders), are the most **common** reported primary cause of DIC in the horse (*Morris 1982, Welch 1992, Topper 1998, Monreal 2000, Dolente 2002, Dallap 2003, Cotovio 2007, Delgado 2009*).

A study evaluating eight **hemostatic parameters** (platelet count, fibrinogen, AT, aPTT, PT, thrombin clotting time, soluble fibrin monomer, and FDPs) in 24 horses presented for colic revealed at least one abnormal parameter in all horses. Nonsurvivors had a much higher average number of abnormal coagulation parameters than in survivors (five versus two) (*Johnstone 1986a*).

This study and other publications support the association of higher mortality with increasing hemostatic dysfunction (*Prasse 1993, Collatos 1995a, Topper 1996, Dallap 2003*). Horses with colitis were eight times more likely to die or be euthanized if they had subclinical DIC, defined as three of six coagulation test results being abnormal (*Dolente 2002*). A study in horses undergoing surgical treatment for large colon volvulus reported seventy percent of these patients to present abnormal results in three of six coagulation tests, and horses with four abnormal results were significantly more likely to be euthanized (*Dallap 2003*).

Furthermore, the magnitudes of prolonged aPTT and PT and decreased AT seem to have **prognostic value** in horses sampled on admission for colic (*Darien 1991, Johnstone 1986b*) or during the first and second day after admission (*Prasse 1993*) and probably reflect the severity of hypercoagulability in these horses. FDPs tend to increase with time in horses hospitalized for colic, and highest values tend to develop in horses with the most severe disease and the poorest prognosis (*Prasse 1993*). This large study including 233 horses with colic also revealed that nonsurvivors had evidence of hypercoagulability most evident in the 24- and 48-hour periods after admission.

Fibrinolytic activity has been reported to increase in patients presented for colic regardless of intestinal ischemia or outcome, suggesting a possible acute phase response secondary to gastrointestinal insult (*Prasse 1993*). Other studies have concluded that fibrinolysis is initially inhibited in response to acute gastrointestinal disease, and that colic horses with severe endotoxemia and DIC present more often hypercoagulation and hypofibrinolysis (*Collatos 1995b, Monreal 2000*).

More recent studies indicate that coagulation profiles may be abnormal in horses with colitis (*Dolente 2002*) or surgical treatment of large colon volvulus before the observation of clinical signs of DIC (*Dallap 2003*). Interestingly, ponies with significantly abnormal coagulation parameters and subsequent death due to experimental small intestinal strangulation did not show specific signs of fulminant DIC (*Pablo 1983*). Excessive hemorrhage has been reported in surgical colic patients, either at surgery or in the immediate postoperative period. The low survival in the group (34%) emphasizes the need for careful coagulation monitoring of surgical colic cases (*Welch 1992*). These examples provide further evidence for the importance of early and accurate identification of horses with coagulation dysfunction.

Future trends

In human and veterinary medicine the problem continues to be **prompt and accurate identification of hemostatic dysfunction,** early enough for a successful intervention. Recent experimental studies have shown that rapid detection and treatment of subclinical coagulopathies reduce morbidity and mortality associated with the development of fulminant DIC (*Wada 1995, Wada 1999*).

It has been suggested that by the time that many of the standard coagulation test results mentioned previously are significantly abnormal, the opportunity for successful treatment could be lost (*Dallap 2004*). Further evaluation is needed to **identify the optimum tests and test cut - offs to include in a DIC scoring scheme adapted for equine species**. The strategy of serial monitoring of routine laboratory tests should be evaluated for clinical diagnosis of non-overt DIC (*Stokol 2010*).

It would also be interesting to **develop a multiparametric model to predict outcome**, including the most predictive tests in the equine patient, and run only those tests that are most useful. It is not easy to say which tests will be most useful due to the variety of primary diseases behind DIC, and therefore future research should determine the coagulation tests with best prognostic value for specific at-risk equine populations.

Recommendations for future research include focusing on earlier identification of at-risk equine patients, **identification of effective anticoagulant or component therapy** to restore physiologic anticoagulant pathways **and evaluation of aggressive therapeutic intervention** (*Dallap 2004*).

Objectives

Objectives

The main objectives of this thesis were:

- ✓ to go further into the study of coagulopathies in horses with colic, with the aim of improving early detection and management of disseminated intravascular coagulation;

- ✓ to evaluate the potential clinical applications of the measurement of plasma D-dimer concentration in horses with colic;

- ✓ to study the evolution of the hemostatic profile in horses with different types of colic;

- ✓ to study the relationship between the hemostatic profile antemortem and the presence of fibrin deposits in tissues found postmortem.

More specifically:

1. to evaluate the relationship of admission plasma D-dimer concentration with the likelihood of survival in horses with several types of colic;

2. to evaluate the association between admission plasma D-dimer concentration and other coagulation profile data with diagnosis and outcome in different acute GI disorders;

3. to assess how the coagulation profile, and specially plasma D-dimer concentration, progresses during hospitalization in horses with different types of colic;

4. to determine the possible influence of administration of antithrombotic therapy, development of thrombophlebitis during hospitalization and of an exploratory laparotomy in the progression of coagulation parameters;

5. to determine the association between the antemortem hemostatic profile, including D-dimer concentration, and the presence of fibrin deposition in different postmortem tissues of horses with gastrointestinal disorders;

6. to evaluate the association between the laboratorial diagnosis of DIC and the presence of histopathologic evidence of DIC;

7. to determine the possible influence of diagnosis in the relationship between the hemostatic profile and postmortem histopathologic evidence of DIC.

Studies

Studies

To fulfill the objectives of this thesis, the following studies were designed and performed:

Study # 1

Association of admission plasma D-dimer concentration with diagnosis and outcome in horses with colic.

Study # 2

Progression of plasma D-dimer concentration and coagulopathies during hospitalization in horses with colic.

Study # 3

Association between necropsy evidence of disseminated intravascular coagulation and coagulation variables before death in horses with colic.

Common Aspects of Materials and Methods
(Studies # 1, 2 & 3)

The aim of this section is to avoid repeating text in each study description in order to describe information about materials and methods that is common to the three studies within this thesis.

About Diagnostic Classification

Diagnostic grouping in the three studies within this thesis was very similar, although not identical, and each study may include some or all the diagnostic groups detailed below.

In each case diagnosis was based on clinical history, complete physical examination including rectal palpation and nasogastric intubation, and results of complementary diagnostic tests (complete blood count, plasma biochemistry, blood gas analysis, abdominal ultrasonography, and peritoneal fluid analysis). Findings of abdominal radiology, exploratory laparotomy and postmortem examination were used for this classification whenever they were performed.

The ***obstructive group*** included all horses with non-strangulating, non-inflammatory disorders of the GI tract, without signs of intestinal devitalization, such as impactions and displacements. The ***medical obstructive group*** included those which resolved with medical therapy (fluid therapy, laxatives and analgesics). The ***surgical obstructive group*** included those that needed surgery because of increasing pain or severe distension (mainly colon displacements).

The ***inflammatory group*** (also named ***enteritis group***) included horses with acute inflammation of small intestine (duodenojejunitis) or large intestine (colitis, typhlitis) or both, diagnosed by compatible clinical signs (i.e. gastric reflux, diarrhea, fever), clinicopathological data (i.e. hypoalbuminemia, acid-base, and electrolyte imbalances) as well as results of complementary examinations (abdominal ultrasound, peritoneal fluid analysis).

The ***ischemic group*** included horses with strangulating GI lesions, such as large colon torsions, small intestinal volvulus, epiploic foramen entrapment, inguinal hernias and intussusceptions. The ***ischemic group without intestinal resection*** included horses in which the affected intestinal portion was considered viable and subsequently had good recovery. Animals were included in the ***ischemic group with intestinal resection*** when any part of the affected bowel needed to be surgically removed.

Finally, the ***peritonitis group*** included horses with severe inflammatory lesions in the abdominal cavity secondary to gastric or intestinal ruptures, as well as septic peritonitis (presence of intra- and extracellular bacteria in peritoneal fluid) caused by bowel devitalization but without rupture, intra-abdominal abscesses and other causes.

About Laboratorial Coagulopathy Classification

Horses were also grouped according to their degree of coagulation dysfunction, defined by the number of abnormal coagulation parameters detected. For the purpose of DIC classification and based on previous studies and reference values in our laboratory (*Monreal 2000, Armengou 2005, Cesarini 2009*), the cut-off values established were as follows: D-dimers >1000 ng/mL, prothrombin time (PT) >15 seconds, activated partial thromboplastin time (aPTT) >65 seconds, antithrombin III (AT) <140% (see **Table 1**). Based on criteria used in the equine literature (*Welch 1992, Monreal 2000, Dolente 2002*) a horse was considered to be in ***laboratorial DIC*** when three or more of these parameters were altered.

Table A. Normal range and values used to diagnose DIC of the 4 hemostatic parameters tested (adapted from *Monreal 2000*).

	Normal range	**Criteria for DIC**
D-dimer (ng/mL)	0-500	>1000
PT (s)	10-12.5	>15
aPTT (s)	36-48	>65
AT (%)	160-210	<140

Other useful hemostatic variables (i.e. platelet count, mean platelet component) were not included because after hours sampling prevented assurance of accurate determination in many cases. Fibrinogen concentration was not included because of its low sensitivity in assessment of coagulopathies in colic horses (*Dolente 2002, Dallap 2003*).

If all four parameters determined were within the defined range horses were classified as presenting a **normal hemostatic profile**.

The diagnosis of **subclinical DIC** was made when horses had ≥3 abnormal parameters in the coagulation profile in the absence of overt clinical signs of coagulopathy. For the purposes of these studies, horses with 1 or 2 abnormal coagulation parameters were considered to have **activation of the coagulation system**.

About Blood Sampling and Processing

Blood samples for determination of coagulation variables were obtained by jugular venipuncture into 5mL tubes containing 3.8% sodium citrate by using a vacutainer system. Immediately after extraction, blood samples were centrifuged at 1,000 x g for 15 minutes, and plasma was frozen at –80ºC until analysis. As reported in previous studies (*Dallap 2003*, *Armengou 2005*), plasma D-dimer concentration, PT, aPTT, and AT were measured in duplicate for all samples.

Plasma D-dimer concentration was determined using an immunoturbidimeter[a] with commercial reagents and controls[b]; PT and aPTT were determined with a semiautomatic coagulometer[c] with commercial reagents and controls[d]. Antithrombin III was determined in a semiautomatic analyzer[e] with a chromogenic kit[f] that measures residual thrombin activity after adding the patient sample to a known quantity of thrombin. Antithrombin activity was expressed as % of that of normal human pooled plasma.

FOOTNOTES

a) Miniquant-1, Biopool, Trinity Biotech, Wicklow, Ireland.

b) Miniquant, Biopool, Trinity Biotech, Wicklow, Ireland.

c) Stago ST4, Stago Diagnostics, Asnières-Sur-Seine, France.

d) Boehringer Mannheim, Mannheim, Germany.

e) Cobas-Bio, Roche, Basel, Switzerland.

f) STA Antithrombin III, Stago Diagnostics, Asnières-Sur-Seine, France.

Study#1

Association of admission plasma D-dimer concentration with diagnosis and outcome in horses with colic

Abstract #1

Background: Coagulopathies detected in horses with gastrointestinal problems seem to be associated with poor outcome. Plasma D-dimer concentration is a sensitive test for assessing coagulopathies.

Hypothesis: Plasma D-dimer concentration tested on admission is related to diagnosis and outcome in horses with colic.

Animals: Four hundred and ninety three horses referred for evaluation of abdominal pain.

Methods: Prospective observational clinical study. Horses were grouped according to diagnosis (medical and surgical intestinal obstructions, ischemic disorders with and without intestinal resection, enteritis, peritonitis), outcome (survivors, nonsurvivors), and number of coagulopathies (normal profile, 1 or 2 coagulopathies, subclinical disseminated intravascular coagulation [DIC]). Blood samples were collected on admission and plasma D-dimer concentration, clotting times (PT and aPTT), and antithrombin activity were determined. Positive likelihood ratios (LR+) were calculated for evaluation of D-dimer cut-off values, which were later tested in a logistic regression model.

Results: Horses with enteritis or peritonitis had significantly ($P < 0.001$) higher plasma D-dimer concentrations and more severe coagulopathies on admission than horses with other diagnoses. Nonsurvivors also had significantly ($P < 0.001$) higher plasma D-dimer concentrations at presentation than did survivors, and those horses with subclinical DIC on presentation had an odds ratio (OR) 8.6 (95% confidence interval [CI], 3.3–22.5, $P < 0.001$) for nonsurvival. Finally, D-dimer concentrations >4,000 ng/mL had a LR+ of 5.9

and an OR 8.8 (95% CI, 4.5–17.1, $P < 0.001$) for nonsurvival.

Conclusion and Clinical Importance: Plasma D-dimer concentration measured on admission can be used to facilitate diagnosis and outcome prediction in horses with colic. A potential cut-off value for nonsurvival was found at approximately 4,000 ng/mL.

Introduction #1

Historically, gastrointestinal (GI) disorders have been the most prevalent cause of coagulation disorders in horses (*Dallap 2004, Monreal 2009*). In horses with acute abdominal pain, coagulation problems are common and usually result in a hypercoagulable state. This can progress to the more severe disseminated intravascular coagulation (DIC), resulting in a subsequent consumption coagulopathy (*Prasse 1993, Dallap 2004&2009, Monreal 2009*). DIC is rarely clinically evident in horses with colic, although it is possible to diagnose subclinical DIC based on compatible clinicopathologic data (*Dolente 2002, Dallap 2003, Monreal 2009*).

There is some evidence that the severity of the host inflammatory response to an inciting event may be extremely critical in the development of coagulopathy and may play an important role in patient outcome (*Marshall 2001*). Ischemic or inflammatory GI problems and other disorders resulting in endotoxemia and severe systemic inflammatory response syndrome more commonly are associated with marked hypercoagulation and may cause DIC (*Morris 1982, Welch 1992, Topper 1998, Monreal 2000, Dolente 2002, Dallap 2003, Cotovio 2007a, Delgado 2009*). A high percentage of horses with colitis (up to 36%) and ischemic lesions (up to 70%) have been reported to be in DIC, especially those disorders that have a poor prognosis, such as large colon volvulus (*Monreal 2000, Dolente 2002, Dallap 2003*). Additionally, approximately 40% of horses with ischemic and inflammatory GI diseases of poor prognosis were found to have massive fibrin deposition in several organs at necropsy consistent with microthrombosis and DIC (*Cotovio 2007a*). Thus, the severity of coagulopathies in horses with GI disorders appears to be related to the diagnosis.

Furthermore, the severity of coagulopathies in horses with GI problems also seems to be associated with poor outcome (*Johnstone 1986b, Prasse 1993, Monreal 2000, Dolente 2002, Collatos 1995b*). In horses with colitis, the odds of survival in a horse with subclinical DIC were decreased 8-fold relative to horses without subclinical DIC (*Dolente 2002*). A study of hemostatic profiles in horses with large colon volvulus that underwent surgery concluded that there was an association between development of coagulopathy and outcome, confirming that horses with 4 of 6 abnormal coagulation tests were more likely to be euthanized, and those with 3 of 6 abnormal tests had a prolonged hospital stay (*Dallap 2003*). Moreover, increases in morbidity and mortality have been associated with alterations in the coagulation and fibrinolysis systems in horses with systemic disease (*Collatos 1995b, Dolente 2002*). Other studies have demonstrated that rapid detection and treatment of subclinical coagulopathies in horses and humans decrease morbidity and mortality associated with development of fulminant DIC (*Wada 1995&1999*).

To make more valid prognostic assessments in colic cases, many authors have evaluated the usefulness of individual variables taken on admission and during hospitalization, including clinical and clinicopathological data (*Parry 1983*, *Johnstone 1986a, Puotunen-Reinert 1986, Orsini 1988, Reeves 1989, Pascoe 1990, Darien 1991, Prasse 1993, Collatos 1995b, Furr 1995, Sandholm 1995, Ihler 2004, Southwood 2009*). Several of these studies included hemostatic parameters and some have been found to be associated with outcome, although results among studies were variable and sometimes contradictory. Common tests to evaluate hemostasis in horses include platelet count, prothrombin time (PT), activated partial thromboplastin time (aPTT), antithrombin activity (AT), fibrinogen, fibrin(ogen) degradation products, and D-dimer concentrations (*Johnstone 1986a, Darien 1991, Prasse 1993, Collatos 1995b, Topper 1998, Monreal 2000, Dolente 2002, Dallap 2003*). Newly available tests, such as mean platelet component (MPC) provided by current CBC analyzers, also have shown to be useful to detect platelet activation (*Segura 2007*). Other hemostatic parameters typically are not available for evaluation of the clinical patient in a hospital setting (*Monreal 2003, Dallap 2004*).

Plasma D-dimer concentration is considered the most sensitive test to diagnose thromboembolic disease and DIC in human medicine, and it also has been shown to be a good predictor of mortality (*Monreal 2003*). In horses, D-dimers have been found to be a useful tool for detection of hypercoagulable states in horses with colic and septic foals during hospitalization (*Monreal 2000, Armengou 2005& 2008*). Furthermore, a marked increase in plasma D-dimer concentration has been found to be associated with ischemic and inflammatory GI disorders, and especially in horses with peritonitis, when determined on presentation (*Armengou 2005, Cesarini 2009, Delgado 2009*).· Some studies also have advocated the usefulness of plasma D-dimer concentration as a good prognostic aid in horses with acute GI problems and recommend its determination for routine screening of colic patients upon admission (*Sandholm 1995, Armengou 2005*).

The objectives of this study were to evaluate (1) the relationship of admission plasma D-dimer concentration with the likelihood of survival in horses with several types of colic, and (2) the association between admission plasma D-dimer concentration and other coagulation profile data with diagnosis and outcome in different acute GI disorders.

Materials and Methods #1

Animals

Horses with colic admitted to the "Fundació Hospital Clínic Veterinari de la Universitat Autònoma de Barcelona" between March 2004 and September 2008 were included in this prospective observational clinical study. Foals younger than 21 days, horses with concomitant inflammatory disorders not related to GI disease (i.e. pneumonia) or liver disease, horses with 2 or more GI disorders of similar severity (i.e. ischemic and inflammatory disorders), and horses with chronic disorders were excluded from the study.

Horses were grouped according to diagnosis in 6 groups, including *medical and surgical intestinal obstructions*, *GI inflammatory conditions, ischemic intestinal problems with or without intestinal resection, and peritonitis*. Horses with ischemic lesions were divided into 2 groups because cases with more severe forms of ischemia and those with prolonged ischemia (>6 hours) are associated with more severe coagulopathies that contribute to the death of the horse (*Welch 1992*). The reader is referred to the previous section "Common Aspects of Materials and Methods" (pages 41-44) for details about definition criteria of this classification.

Horses also were grouped according to outcome: survivors (horses that were discharged from the hospital), nonsurvivors (horses that died during hospitalization because of progressive worsening of disease or that were euthanized based on poor prognosis), and the financial restraint group (horses that did not receive the suitable treatment for financial or personal reasons). Horses that were either euthanized or left the hospital without medical consent because of economic restraints were not considered in outcome analysis.

Finally, according to the degree of coagulopathy presented, horses were classified as having a *normal hemostatic profile*, having *activation of the coagulation* system or being in *subclinical DIC* (see section on “Common Aspects of Materials and Methods“ (pages 41-44) for details).

Additionally, a group of 30 healthy horses was used as controls, and included horses in regular training from a riding school near the Teaching Hospital, controlled blood donors, and healthy mares (41 week postpartum) that had accompanied their hospitalized sick foals.

Blood Sampling and Measured Parameters

On admission, citrated blood samples were obtained by jugular venipuncture, centrifuged and plasma was frozen at –80ºC until analysis. Plasma D-dimer concentration, PT, aPTT, and AT were determined as previously described (see section on “Common Aspects of Materials and Methods” (pages 41-44) for details). Clotting times and AT were determined to complement plasma D-dimer concentrations and to allow classification of coagulation dysfunction.

Statistical Analysis

Results were expressed as frequencies and percentages for qualitative variables and median (interquartile range) for quantitative variables. Univariate inferential analysis was performed by Fisher’s exact test for categorical data or the Mann-Whitney U test for quantitative variables. D-dimer concentration was assessed by means of the positive likelihood ratio (LR+) estimation for dying/being euthanized, defined as sensitivity/(1-specificity), to obtain different cut-off values. In general, values of LR+ higher than 5 indicate a moderate increase in the likelihood of the disease, and values higher than 10 indicate a large and often conclusive likelihood.

An interval validation of these cut-off values also was performed by means of logistic regression models. Several multivariate models were evaluated by the cut-off values obtained by LR+, adding 1 of the other 3 hemostatic parameters tested (PT, aPTT, or AT) in this statistical study. D-dimer concentration was included as a fixed factor in all models for different cut-off values (2,000, 3,000, 4,000, 5,000, and 5,500 ng/mL) in order to assess the influence of the 4 hemostatic parameters as covariables. An estimation of the risk of death by number of abnormal coagulation parameters was performed by a univariate logistic regression model.

P values ≤ 0.05 were considered statistically significant. With the aim of not inflating type I error rate in subtype diagnostic analysis, comparisons with the control group were only performed by global type (obstructions or ischemias) when non significant relations were observed between subtype of diagnosis (medical versus surgical obstructions, and ischemias with versus without intestinal resection). Statistical analysis was performed by the SPSS 15.0 package (Chicago, IL, USA).

Results # 1

Of all horses admitted for colic during the study period, 493 horses met the inclusion criteria and were included in the study. The sex distribution was 174 females (35.5%), 139 intact males (28.4%), and 177 geldings (36.1%). No sex information was available for 3 horses. The median age (interquartile range) of the population was 8 years (5–12 years). No age information was available for 9 horses. One hundred seventy-four horses (35.6%) were Andalusians, 122 horses (24.9%) were crossbred, 28 (5.7%) Arabians, 26 (5.3%) Warmbloods, 24 (4.9%) ponies, 14 (2.9%) and Draft breeds, and the remaining 97 horses were a mixture of different breeds. No breed information was available for 4 horses.

Diagnoses included 229 horses (46.5%) with medical obstructions, 74 (15%) surgical obstructions, 74 (15%) inflammatory problems, 33 (6.7%) ischemic lesions without intestinal resection, 51 (10.3%) ischemic lesions with intestinal resection, and 32 (6.5%) horses with peritonitis.

Of the 493 horses, 387 (78.5%) survived, 80 (16.2%) died or were euthanized because of poor prognosis, 23 (4.7%) were euthanized because of financial constraints, and 3 horses (0.6%) left the hospital against medical advice. The outcome of horses with different diagnoses is shown in **Table 1.1**.

Hemostatic Profile in the Different Diagnostic Groups

Results of coagulation profile data by diagnostic group are presented in **Tables 1.2 and 1.3**. On admission, D-dimer concentration was markedly and significantly ($P< 0.001$) higher in inflammatory diseases (enteritis and peritonitis groups) compared with controls.

Table 1.1. Outcome distribution of horses with different diagnoses.

	Survivors (n= 387)	**Non-survivors** (n= 80)	**Financial restraints** (n= 26)
Medical obstructions	211 (54.5%)	2 (2.5%)	16 (61.5%)
Surgical obstructions	67 (17.3%)	7 (8.8%)	0 (0.0%)
Ischemia without intestinal resection	25 (6.5%)	4 (5.0%)	4 (15.4%)
Ischemia with intestinal resection	23 (5.9%)	25 (31.3%)	3 (11.5%)
Enteritis	55 (14.2%)	17 (21.3%)	2 (7.7 %)
Peritonitis	6 (1.6%)	25 (31.3%)	1 (3.9%)
	100%	100%	100%

Numbers correspond to absolute number of horses regarding the outcome and percentages to the relative proportion in the same outcome group and within the different diagnostic groups.

Table 1.2. Coagulation profile results obtained on presentation among the different diagnostic groups.

	Controls (***n*** = 30)	**Medical Obstructions** (*n* = 229)	**Surgical Obstructions** (*n* = 74)	**Ischemic without resection** (*n* = 33)	**Ischemic with resection** (*n* = 51)	**Enteritis** (*n* = 74)	**Peritonitis** (*n* = 32)
D-dimer concentration *(ng/mL)*	695.0 [586;742]	308.0* [133;923]	419.5* [168.5;919.5]	521.0 [135;1,212]	1,241.8 [360;2,687]	1,048.3* [435;2,711]	4,204.0* [1,652.5;5,923]
PT *(sec)*	11.05 [10.85;11.4]	12.1* [11.3;13.8]	12.3* [11.2;14]	12.7* [11.6;13.8]	12.9* [11.6;14.6]	13.6* [12.1;15.6]	13.9* [13.0;15.1]
aPTT *(sec)*	39.0 [36.9;41.2]	50.2* [44.7;59.7]	50.9* [44.8;60.1]	49.3* [46.0;58.0]	51.3* [44.2;61.6]	55.3* [49.5;64.1]	54.7* [46.0;61.5]
AT *(%)*	188.2 [172;211]	214* [196;234]	220* [198;244]	208 [181;230]	201 [177;220]	192 [166;214]	164 [144;204]

Results are expressed as median [interquartile range: P25th; P75th]. * Statistically significant (P<0.001) when compared to the control group.

Table 1.3. Distribution of coagulation profile data for different cut-off values (normal or abnormal values) in the main diagnostic groups.

Cut-off values	**Controls** (n = 30)	**Medical Obstructions** (n = 229)	**Surgical Obstructions** (n = 74)	**Ischemic without resection** (n = 33)	**Ischemic with resection** (n = 51)	**Enteritis** (n = 74)	**Peritonitis** (n = 32)
D-dimer concentration (ng/mL)							
< 1,000	29 (96.7%)	181 (79%) *	57 (77%) *	23 (69.7%) *	25 (49%) *	38 (51.4%) *	6 (18.7%) *
> 1,000	1 (3.3%)	48 (21%) *	17 (23%) *	10 (30.3%) *	26 (51%) *	36 (48.6%) *	26 (81.3%) *
> 2,000	0 (0%)	22 (10.3%)	10 (13.5%)	3 (9.7%) #	16 (33.3%) * #	26 (36.1%) *	20 (66.7%) *
> 3,000	0 (0%)	10 (4.7%)	7 (9.5%)	3 (9.7%)	10 (20.8%) *	12 (16.7%) *	17 (56.7%) *
> 4,000	0 (0%)	7 (3.3%)	6 (8.1%)	3 (9.7%)	7 (14.6%) *	7 (9.7%)	16 (53.3%) *
> 5,000	0 (0%)	2 (0.9%) #	4 (5.4%) #	1 (3.2%)	2 (4.2%)	3 (4.2%)	9 (30%) *
PT (seconds)							
< 15	30 (100%)	198 (86.5%) *	63 (85.1%) *	29 (87.9%)	44 (86.3%) *	56 (75.7%) *	24 (75%) *
> 15	0 (0%)	31 (13.5%) *	11 (14.9%) *	4 (12.1%)	7 (13.7%) *	18 (24.3%) *	8 (25%) *
aPTT (seconds)							
< 65	30 (100%)	197 (86%) *	64 (86.5%)	29 (87.9%)	41 (80.4%) *	59 (79.7%) *	26 (81.3%) *
> 65	0 (0%)	32 (14%) *	10 (13.5%)	4 (12.1%)	10 (19.6%) *	15 (20.3%) *	6 (18.8%) *
AT (%)							
> 140	30 (100%)	226 (98.7%)	71 (95.9%)	33 (100%)	48 (94.1%)	66 (89.2%)	27 (84.4%)
< 140	0 (0%)	3 (1.3%)	3 (4.1%)	0 (0%)	3 (5.9%)	8 (10.8%)	5 (15.6%)

Results show the number of horses and the percentage in regard to the diagnosis group.

* Statistically significant when compared to controls.

Statistically significant when compared between diagnostic subgroups (for the obstructive and the ischemic groups).

Despite being also increased in the ischemic group that needed resection, concentrations did not reach significant differences. Clotting times were mildly but significantly prolonged in all colic groups, and AT showed mild differences in both obstructive groups when compared with controls. Comparing horses with medical versus surgical obstructive problems, no significant differences were found between them in any of the hemostatic parameters determined.

Despite that fact, when specific cut-off values were compared, only 0.9% of medical obstructive cases had plasma D-dimer concentrations on admission >5,000 ng/mL, compared with 5.4% in the surgical group (P=0.04). Comparing horses with ischemic disorders, a tendency toward significance (P<0.06) was seen for higher plasma D-dimer concentration on admission in ischemic cases that needed intestinal resection compared with those in which this procedure was not necessary (**Table 1.3**). Furthermore, when specific cut-off values were compared, most (90.3%) ischemic cases without intestinal resection had plasma D-dimer concentrations on admission ≤2,000 ng/mL, compared with 66.7% in the resection group (P=0.017). Differences in other parameters were not significant.

The distribution of horses by number of altered parameters in the coagulation profile by diagnostic group is shown in **Table 1.4.** Horses with enteritis, ischemic lesions that needed intestinal resection and peritonitis had a higher number of coagulopathies, and clinicopathological DIC already was diagnosed on admission in horses with enteritis and peritonitis (10.8 and 9.4%, respectively).

Hemostatic Profile in the Different Outcome Groups

Results of clotting profile data by outcome groups are shown in **Table 1.5**.

Table 1.4. Distribution of horses by number of coagulopathies detected on presentation in the different diagnostic groups.

Number of abnormal hemostatic parameters (out of 4)	**Medical Obstructions** (n = 229)	**Surgical Obstructions** (n = 74)	**Ischemic without resection** (n = 33)	**Ischemic with resection** (n = 51)	**Enteritis** (n = 74)	**Peritonitis** (n = 32)
Normal profile	157 (68.3%)	44 (59.5%)	20 (60.6%)	18 (35.3%)	27 (36.5%)	4 (12.5%)
Activated coagulation system (1 or 2 abnormal parameters)	61 (26.6%)	29 (39.2%)	12 (36.4%)	31 (60.8%)	39 (52.7%)	25 (78.1%)
Subclinical DIC (= 3 abnormal parameters)	11 (4.8%)	1 (1.4%)	1 (3%)	2 (3.9%)	8 (10.8%)	3 (9.4%)

The numbers correspond to absolute number of horses and percentages to the relative proportion in the diagnostic groups.

Table 1.5. Results of the coagulation profile in the main outcome groups (survivors and non-survivors).

	Survivors (n = 387)	**Non-survivors** (n = 80)	**Cut-off values**	**Survivors**	**Non-Survivors**	**OR (95% CI)** ***P*-value**
D-dimer concentration	383	2,304*	= 1,000	74.2%	35.0%	5.33 (3.19 to 8.9)
(ng/mL)	[143; 1,112]	[733; 4,489]	> 1,000	25.8%	65.0%	P < 0.001
			= 4,000	94.8%	67.5%	8.78 (4.52 to 17.05)
			> 4,000	5.2%	32.5%	P < 0.001
PT	12.3	13.8*	< 15	86%	76.3%	1.9 (1.1 to 3.5)
(sec)	[11.3; 13.9]	[12.0; 15.1]	> 15	14%	23.8%	P = 0.03
aPTT	50.7	54.7	< 65	85.5%	80%	1.5 (0.8 to 2.7)
(sec)	[45.3; 60.7]	[44.4; 61.0]	> 65	14.5%	20%	P = 0.21
AT	212	192*	> 140	98.4%	81.3%	14.7 (5.5 to 39.1)
(%)	[190; 234]	[144; 212]	< 140	1.6%	18.8%	P < 0.001

Results are expressed as median [interquartile range: P25th; P75th]. Distribution (percentage) of these coagulation data for different cut-off values in the main outcome groups is shown, as well as the OR for the corresponding cut-off value. * Statistically significant (P<0.001) when compared to survivors for quantitative results.

Plasma D-dimer concentration was marked and significantly (P<0.001) higher in dead/euthanized horses than in discharged horses. Furthermore, 74.2% of survivors had plasma D-Dimer concentrations within the normal range (<1,000 ng/mL) compared with 35% of nonsurvivors (odds ratio [OR], 5.33; 95% confidence interval [CI], 3.19–8.9, P<0.001). PT also was significantly (P<0.001) prolonged in nonsurvivors, and the percentage of horses with PT >15 seconds also was higher in nonsurvivors (OR, 1.9; 95% CI, 1.1–3.5, P=0.03). Antithrombin activity was significantly (P<0.001) lower in dead/euthanized horses than in discharged ones, and significantly more animals with poor prognosis had AT < 140% (OR, 14.7; 95% CI, 5.5–39.1, P<0.001). Differences were not significant for aPTT.

Regarding the type of coagulopathy, there were significantly (P=0.009) more horses with clinicopathological DIC (>3 abnormal hemostatic parameters) upon presentation in nonsurvivors (11.3%) than in survivors (3.6%) (**Table 1.6**).

Using the univariate logistic regression model, horses with 1 or 2 coagulopathies on presentation were more likely to die (OR, 5.3; 95% CI, 3.0–9.4, P<0.001). When horses had clinicopathological evidence of DIC (3 or 4 of 4 abnormal parameters) on presentation, the OR value for mortality increased to 8.6 (95% CI, 3.3–22.5, P<0.001).

Table 1.6. Distribution of horses by number of coagulopathies detected on presentation in the different outcome groups.

Number of abnormal hemostatic parameters on admission (out of 4)	**Survivors** (n = 387)	**Non-survivors** (n = 80)	**OR** (95%CI)	***P*-value**
Normal profile	240 (62.0%)	18 (22.5%)	Baseline	
Coagulation system activated (1 or 2 abnormal parameters)	133 (34.4%)	53 (66.3%)	5.3 (3.0 to 9.4)	< 0.001
Subclinical DIC (≥ 3 abnormal parameters)	14 (3.6%)	9 (11.3%)	8.6 (3.3 to 22.5)	< 0.001

The numbers correspond to absolute number of horses and percentages to the relative proportion in the outcome groups.

Results of the Positive Likelihood Ratios and Logistic Regression Models

D-dimer concentrations of approximately 4,000 ng/mL showed a LR+ value of 5.9 for dying/being euthanized. No optimal interval of results to suggest potentially useful cut-off values was identified for the remaining parameters (results not shown). The logistic regression model including plasma D-dimer concentration (crude analysis) resulted in an OR of 8.78 (95% CI, 4.52–17.05, P<0.001) for nonsurvival when values were approximately 4,000 ng/mL. Adjusted OR for D-dimer concentration (>4,000 ng/mL) by mean PT (>15 seconds), aPTT (>65 seconds), or AT (<140%) did not show substantial changes from crude OR evaluation of D-dimer analysis. Only AT values <140% maintained a significant effect in the presence of the D-dimer factor.

Discussion # 1

The most common coagulopathy in horses with colic is a hypercoagulable state that can progress to DIC. The intensity of this coagulopathy will depend on the severity and duration of the GI lesion, with ischemic and inflammatory problems and peritonitis being most frequently affected by coagulopathies (*Monreal 2000&2003*). Furthermore, the severity of coagulopathies in horses with GI problems also seems to be associated with poor outcome (*Johnstone 1986b, Prasse 1993, Monreal 2000, Dolente 2002, Collatos 1995b*). In the present study, plasma D-dimer concentration was related to diagnosis and prognosis, which could help to differentiate horses with enteritis and peritonitis from other diagnoses, and especially to detect horses with poor outcome. Furthermore, D-dimer concentration was the most sensitive coagulation parameter for these diagnostic and prognostic purposes of the parameters tested in the present study.

Regarding diagnosis, results of this study indicate that patients with enteritis or peritonitis had significantly higher D-dimer concentration when tested on presentation, AT was significantly lower and alterations in hemostatic parameters were more severe. These 2 diagnostic groups also included a higher percentage of horses with subclinical DIC on admission. In the literature, inflammatory and ischemic conditions are the diagnoses most often associated with severe coagulopathies, especially when diagnosed during hospitalization (*Welch 1992, Monreal 2000, Dolente 2002, Dallap 2003*). In the present study, however, severe coagulopathies were detected upon presentation. In horses with colic and in septic foals, the inflammatory response, and consequently activation of the coagulation system, can be more severe some hours after presentation (*Prasse 1993, Dallap 2003, Armengou 2005, Cesarini 2009*) which could explain why abnormalities of the coagulation profile detected on admission usually are milder. In addition, in the present study D-dimer concentration in ischemic disorders taken as a whole was not significantly higher on admission, although a tendency toward significance (P = 0.06) was present when comparing horses with or without resection, with D-dimer concentration higher in horses that needed enterectomy. Previous studies also suggest that D-dimer concentrations in ischemic colicky horses tend to increase early after admission (*Byars 1982, Cesarini 2009*). Thus, the lack of significance in the group that needed intestinal resection could be associated with the fact that samples were taken too early in the ischemic process.

Regarding diagnosis, when specific concentrations of D-dimers were analyzed, one third of ischemic horses with intestinal resection had plasma D-dimer concentrations on admission of >2,000 ng/mL, compared with just 10% of horses in which intestinal resection was not necessary. This additional information can help the clinician determine prognosis and treatment when confronted with such types of colic. In contrast, in obstructive colics there were significantly more horses with plasma D-dimer concentrations on admission >5,000 ng/mL in the surgical group compared with the medical group. Most horses in both groups (94.6 and 99.1%, respectively), however, had lower concentrations, which limits the applicability of this information in a clinical setting. Clotting times also showed significant differences when compared with controls, but not between medical and surgical obstructions or ischemic problems with or without resection. Finally, AT did not show significant differences for the cut-off values applied.

Enteritis and peritonitis were the diagnoses with the higher prevalence of subclinical DIC upon admission and, together with ischemic lesions (especially those requiring resection), had the highest number of patients with activation of the coagulation system. These findings are in accordance with previous studies (*Monreal 2000, Dallap 2003, Delgado 2009*). Thus, these severe inflammatory conditions seem to be linked to severe coagulopathies, which are already detected on admission when using sensitive tests. Other diagnoses (such as ischemic disorders) would need to be retested after presentation or by use of a larger coagulation profile to better assess the associated coagulopathy.

In general, diagnoses corresponding to more severe disorders, most commonly associated with nonsurvival, also had the highest percentage of horses with the highest D-dimer concentrations.

Several studies have attempted to elucidate the relation of coagulation abnormalities during colic with fatality (*Byars 1982, Johnstone 1986b, Darien 1991, Prasse 1993, Collatos 1995b, Sandholm 1995, Ihler 2004*). One of the objectives of this study was to evaluate the usefulness of admission results of several hemostatic parameters to predict the likelihood of survival. In this study, plasma D-dimer concentrations on presentation were significantly higher, PT significantly prolonged and AT significantly lower in nonsurvivors than in survivors. However, while most nonsurvivors had markedly increased D-dimer concentrations (>1,000 ng/mL), a lower percentage of nonsurvivors had PT and AT results above the reference range (>15 seconds and <140%, respectively). Thus, according to these results, D-dimer concentration seems to be more associated with prognosis than the remaining coagulation variables tested. Other studies in horses with colic also found higher D-dimer concentrations in nonsurvivors (*Sandholm 1995*), as well as prolonged PT and decreased AT, thus confirming these results (*Holland 1986, Johnstone 1986b, Prasse 1993, Topper 1998, Dolente 2002, Dallap 2003*).

Horses with plasma D-dimer concentrations on admission >1,000 ng/mL and >4,000 ng/mL were found to be 5.33 and 8.78 times more likely to die, respectively. This information can be useful to the clinician to predict outcome in a case of colic. Furthermore, horses with AT results below 140% had 14.7 times more probability of nonsurvival. Nevertheless, results of AT are rarely below this level, even in severe colics, and so this information has little clinical value. In contrast, PT did not show a good predictive value for nonsurvival (OR=1.9). Thus, following our results, D-dimer concentration could have better prognostic value than the other coagulation parameters tested.

When the coagulopathies were assessed for outcome, there were significantly more horses with subclinical DIC (>3 altered parameters) in the group of nonsurvivors. Furthermore, horses had 8.6 times more probability to die if they were in subclinical DIC, and 5.3 times more probability if they only had 1 or 2 abnormal coagulation parameters. This finding is not surprising because the literature reports worse outcomes in horses with GI problems associated with a high number of coagulopathies and DIC (*Johnstone 1986a, Prasse 1993, Collatos 1995a, Dallap 2003*). Postmortem studies also found higher fibrin deposition scores in different organs of horses with colic diagnoses more commonly associated with DIC (*Cotovio 2007ab*). Nevertheless, previous results complementary with this study confirm that alterations of the coagulation profile in horses that survived tend to normalize during hospitalization regardless of the diagnosis (*Cesarini 2009*).

The results of this study have confirmed that changes in hemostasis are frequent in severe GI disorders of horses. D-dimer concentration determined on presentation has a clear relationship with outcome. Results of the LR+ in this population of colic horses confirmed that plasma D-dimer concentration is a sensitive parameter, with concentrations on admission >4,000 ng/mL indicating a moderate to marked increase in the likelihood of dying or being euthanized. This analysis is very similar to ROC curves and area under curve calculations and can be used for equivalent purposes to determine the diagnostic and prognostic utility of a test in different diseases. The LR+ for death used in the present study has the advantage of offering the same optimal cut-off point as does graphical evaluation of the ROC curve, but also considers the fitness of the cut-off point. Furthermore, the logistic regression models combining AT and plasma D-dimer concentration showed the best LR+ with D-dimer concentrations to be approximately 4,000 ng/mL. Therefore, based on this study, if a D-dimer cut-off concentration of 4,000 ng/mL is used on admission, the likelihood ratio of death independently of diagnosis is approximately 6:1 (i.e. 6 horses with D-dimer concentration >4,000 ng/mL are likely to die for each horse with D-dimer concentration >4,000 ng/mL that survives). In contrast, other studies have not found that D-dimer concentrations provide any additional information in explaining outcome when tested on admission, but they used less accurate methods in a smaller population of horses (*Dallap 2003, Ihler 2004*).

One possible limitation of this study is that the number of hemostatic parameters determined was low, and did not include platelet count or other commonly tested parameters characterized by their low sensitivity. Nevertheless, the parameters determined in this study are those currently used in humans and dogs to evaluate coagulopathies and constitute the basis of DIC scoring systems in these species or are used to increase diagnostic sensitivity (*Bakhtiari 2004, Toh 2005, Wiinberg 2010*). With such a low number of parameters tested, identification of coagulopathies and diagnosis of DIC could be limited. However, the percentage of coagulopathies and diagnosis of DIC finally detected in the present study was similar to what has been reported in other studies in which more hemostatic parameters were tested.

In conclusion, the present study confirmed that when a simple hemostatic profile that includes D-dimer concentration was assessed on admission in horses with colic, the most severe coagulopathies were observed in horses with the most severe forms of GI disorders (i.e. inflammatory conditions, ischemic intestinal problems that needed intestinal resection, peritonitis) and in nonsurvivors. Furthermore, the diagnoses with higher percentage of nonsurvival also had more horses with high concentrations of D-dimers. Moreover, this study also supports the usefulness of D-dimer concentration tested on admission to predict outcome in horses with GI disorders, especially when >4,000 ng/mL. Additional studies are warranted using this cut-off value in a different population of horses to confirm its possible diagnostic and prognostic value.

Study#2

Progression of plasma D-dimer concentration and coagulopathies during hospitalization in horses with colic

Abstract #2

Background**:** In horses with gastrointestinal problems significant coagulation alterations may appear or worsen during the first days of hospitalization or after surgery. A follow up of plasma D-dimer concentration could help clinicians to early recognize disseminated intravascular coagulation (DIC) in these patients.

Hypothesis**:** Plasma D-dimer concentration will progress differently depending on the diagnosis of the gastrointestinal problem and could be influenced by the presence of thrombophlebitis, administration of antithrombotic therapy and abdominal surgery.

Animals**:** Horses admitted for colic receiving a suitable treatment and remained hospitalized more than 48 hours were initially considered. Due to the limited follow up in most dead horses, only data from 91 discharged horses was finally analyzed.

Methods**:** Prospective observational clinical study. Animals were classified by diagnosis in medical obstructions, surgical obstructions, inflammatory problems and ischemic lesions. At least three blood samples were obtained from each horse (Admission, 24-48h (medical cases) or after surgery (surgical cases) and Discharge). For each sample, plasma D-dimer concentration, prothrombin time, activated partial thromboplastin time, antithrombin activity and the presence of laboratorial DIC were determined. Information about antithrombotic treatment and development of thrombophlebitis was recorded. General Estimating Equation Models were used to study the association of diagnosis and the influence of receiving

antithrombotic therapy or having thrombophlebitis on the development of coagulopathy.

Results**:** Plasma D-dimer concentration was significantly lower in medical obstructive colics compared to other diagnoses at all time points. Most inflammatory colics presented coagulopathies already on admission, but tended to normalize before discharge. Both surgical colics (obstructions and ischemic) developed a marked hypercoagulability postoperatively that was not related to diagnosis.

Conclusion and Clinical Importance**:** Medical obstructive colics had less severe coagulopathies and lower plasmatic D-dimer concentrations throughout hospitalization compared to other diagnosis. At 24-48h of hospitalization the hemostatic profile can be very different when compared to admission values. Most inflammatory colics presented coagulopathies already on admission. Independently of diagnosis, horses requiring exploratory celiotomy trended towards hypercoagulability. Thromboprophylaxis could be considered in both clinical situations.

Introduction #2

Activation of the coagulation system resulting in a hypercoagulable state is a frequent finding in horses with gastrointestinal disorders (*Johnstone 1986a, Darien 1991, Welch 1992, Prasse 1993, Collatos 1995, Monreal 2000, Dolente 2002*). Coagulopathies in horses can lead to serious thrombotic complications, such as disseminated intravascular coagulation, or pulmonary thromboembolism, but more commonly they contribute to less serious thrombotic events, such as thrombophlebitis (*Welch 1992, Morris 1989, Traub-Dargatz 1994, Dolente 2005, Norman 2008*). The intensity of the coagulopathy will depend on the severity and duration of the GI lesion as well as on the degree of activation of the host inflammatory response. Severe inflammatory problems (including peritonitis) and ischemic disorders are the most frequently affected by coagulopathies (*Morris 1982, Welch 1992, Topper 1998, Monreal 2000, Dolente 2002, Dallap 2003, Cotovio 2007, Delgado 2009*). An association between the severity of these coagulopathies in horses with GI diseases and a lower survival rate also has been established (*Johnstone 1986b, Prasse 1993, Collatos 1995b, Dallap 2003, Cesarini 2010, Dunkel 2010*).

However, most changes in coagulation parameters detected in horses with acute GI disease have been reported from single samples, usually taken upon admission, without consideration of therapeutic intervention and the progression over time (*Johnstone 1986b, Holland 1986, Darien 1991, Welles 1991, Prasse 1993, Collatos 1995b*). Interestingly, some studies that have prolonged the evaluation of hemostasis during several days after admission reported that significant changes frequently become apparent only after 24-72 hours of hospitalization (*Holland 1986, Dolente 2002, Dallap 2003, Feige 2003*). These coagulation alterations normally increase during the first days of hospitalization (medical cases) and after surgery (ischemic cases), but they usually return to reference ranges when horses are discharged (*Dallap 2003, Feige 2003a*). Furthermore, there is also indirect evidence suggesting that not only the colic disease itself, but also the event of surgery results in a marked activation of the coagulation system (*Collatos 1995b, Moore 1995, Feige 2003a*).

D-dimer is a specific product resulting from the lysis of cross-linked fibrin by the fibrinolytic system. Increased D-dimer concentrations in plasma indicate activation of fibrinolysis because of excessive formation of fibrin within the vascular tree (*Monreal 2003*). In human medicine, D-dimer is considered a very sensitive indicator of DIC, deep venous thrombosis, pulmonary embolism, and catheter-associated venous thrombosis. Furthermore, D-dimer is also used in humans to identify patients at risk for DIC and their response to antithrombotic therapy (*Tripodi Adam 2009, 2011*). Several studies in dogs and horses support the use of D-dimers as a very sensitive test to diagnose hypercoagulative states, thromboembolic disease and DIC (*Monreal 2000, Nelson 2003, Armengou 2008 Delgado 2009, Cesarini 2010*). In horses, marked increases of plasma D-dimer concentration determined on presentation have been associated to ischemic and inflammatory GI disorders, especially peritonitis, as well as to a poor prognosis (*Sandholm 1995, Cesarini 2010, Delgado 2009*). This test could help clinicians to recognize critically ill patients with DIC and thromboembolic disease which would benefit from early antithrombotic therapy, so that mortality in these patients could be reduced (*Monreal 2003*).

Thus, the main objectives of this study were: 1) to assess how the coagulation profile, and specially plasma D-dimer concentration, progresses during hospitalization in horses with different types of colic; 2) to determine the possible influence of administration of antithrombotic therapy, development of thrombophlebitis during hospitalization and of an exploratory laparotomy in the progression of coagulation parameters.

Materials and Methods #2

Animals

In this prospective observational clinical study, horses admitted for colic to the "Fundació Hospital Clínic Veterinari de la Universitat Autònoma de Barcelona", between March 2004 and September 2008, were considered. Initially, all horses that received a suitable treatment (either medical or surgical) and remained hospitalized more than 48 hours were considered. Finally, only horses discharged in good condition were included in the statistical analysis. Horses that died or were euthanized because of poor prognosis were not included because follow up (multiple samples) was only possible in a few of them. For this reason, only descriptive data of non-surviving horses will be shown in the results section.

Exclusion criteria in this study included: all foals younger than 21 days, horses with concomitant inflammatory disorders which were not related with the GI disease (i.e. pneumonia) or with liver disease, horses with 2 or more GI disorders of similar severity (such as ischemic plus inflammatory disorders), horses with chronic disorders and horses that did not receive the suitable treatment for economical constraints.

Horses were classified according to diagnosis in 4 groups: (1) *medical obstructive*, (2) *surgical obstructive*, (3) *inflammatory* and (4) *ischemic* disorders. The reader is referred to the previous section "Common Aspects of Materials and Methods" (pages 41-44) for details about definition criteria of this classification.

Finally, horses were also grouped according to the number of abnormalities detected in the hemostatic profile as being *without coagulopathy*, having *activation of the coagulation system* (1 or 2 abnormalities) or being in *subclinical DIC* (≥3 abnormal parameters). See the section on “Common Aspects of Materials and Methods” (pages 41-44) for further details.

Blood Sampling and Measured Parameters

A first blood sample was taken on admission, before receiving any therapy, in all animals. A second blood sample was taken at 24-48 hours after admission in patients with medical problems and between up to 20h after the end of exploratory laparotomy in surgical cases (which in most cases was approximately 24-36 hours after admission). Finally, a third sample was taken in all cases before being discharged from the hospital.

Citrated blood samples were obtained by jugular venipuncture, centrifuged and plasma was frozen at –80ºC until analysis. Plasma D-dimer concentration, PT, aPTT, and AT were determined as previously described (see section on “Common Aspects of Materials and Methods” (pages 41-44) for details).

Anticoagulant therapy and other treatments received during hospitalization

Information about treatment or not with LMWH during hospitalization was recorded in all cases. If horses received LMWH, the treatment administered in all cases was 50 IU/kg dalteparin (Fragmin®, Kabi Pharmacia AB, Sweden), SC, q24h for 3 days.

The *medical obstructive group* received IV or enteral fluids as main treatment. Few cases needed low doses of analgesics. None of these horses received LMWH. The *surgical obstructive group* usually received medical treatment with analgesics and IV fluids until the attending clinician established the need for an abdominal laparotomy. Treatments after surgery included IV fluids, antimicrobials, and analgesics/NSAIDs when necessary. In this group, based on surgeon's criteria, some horses received LMWH therapy.

In the *ischemic group*, a resection of the affected intestine was performed or not depending on subjective assessment of viability by the attending surgeon. Treatments after surgery included: intravenous fluids, antimicrobials, analgesics/NSAIDs and prokinetics when necessary. In this group, most horses received LMWH therapy after surgery following surgeon's criteria, based on the severity of the ischemic lesion and the need for intestinal resection. In the *inflammatory group*, horses were treated with IV fluids, prokinetics, analgesics and others. Most of these horses received LMWH therapy.

Development of jugular thrombophlebitis during hospitalization

Retrospectively, the presence (or not) of thrombophlebitis at each time point (admission, 24-48h, and discharge) was documented. Horses were considered to have thrombophlebitis if they had the presence of a thrombus adhered to the vein evident by ultrasonographic examination and/or compatible clinical signs, such as palpable thickening of the vein wall, pain, and swelling, poorly distended vein when held off, or abnormal resistance to catheter flow (*Divers 2003*).

Statistical Analysis

Initially, both quantitative and qualitative descriptive analyses of data were performed. For the purpose of classifying data in the qualitative analysis, the studied parameters were considered abnormal when D-dimer concentration was >1,000 ng/mL, PT>15 seconds, aPTT>65 seconds, and AT<140%, based on previous studies and reference values of our laboratory (*Monreal 2000, Armengou 2008, Cesarini 2009& 2010*).

Results were expressed as frequencies and percentages for qualitative variables. For quantitative variables, median was used as central estimation of values and interquartile range (percentiles 25^{th} and 75^{th}) were used as measurement of precision of results. Data were ranked and three General Estimating Equation (GEE) models were used to study respectively the association between diagnosis type, diagnosis type adjusted for the presence of thrombophlebitis and diagnosis type adjusted for the treatment or not with LMWH on the development of coagulopathy. GEE models are a good methodology to analyze longitudinal data and sensitive enough to estimate intra-subject variability. For this purpose in this study we used an 'unstructured' matrix and 'horse' as intra-subject factor. An 'unstructured' covariance is often used to model the within-subject errors in the analysis because no assumptions are made about the within-subject variability.

Furthermore, a non-parametric analysis was used to study differences between diagnoses at each time point and a U Mann-Whitney analysis to study the presence of coagulopathy (normal, activation and subclinical DIC) of medical obstructive *vs*. surgical obstructive colics over time. P values < 0.05 were considered statistically significant. Statistical analysis was performed by the SPSS (IBM) 20.0 package (Chicago, IL, USA).

Results #2

Animals

A total of 640 horses were admitted for colic between March 2004 and September 2008, from which 290 met the inclusion criteria. From these, 245 horses remained hospitalized more than 48h and were discharged in good condition, but only 91 had enough parameters determined at all sampling times, and were finally included in the statistical analysis of the study.

The gender distribution was 34 females (37.4%), 26 intact males (28.6%) and 31 geldings (34.1%). Thirty seven (40.7%) horses were Andalusians, 17 (18.7%) crossbred, 9 (9.9%) Arabians, 6 (6.6%) French Saddlebreds, 6 (6.6%) ponies, 2 (2.2%) draft breeds and the other 14 (15.4%) were a mix of other breeds. Diagnoses included 26 (28.6%) medical obstructions, 24 (26.4%) surgical obstructions, 22 (24.2%) inflammatory problems and 19 (20.9%) ischemic lesions.

Ninety four horses with colic admitted during the study period had a poor outcome, but only 31 died/were euthanized because of poor prognosis (no economical constraints) and had multiple samples taken (to allow a certain follow up). From these 31 horses, only 13 horses had enough parameters determined at all sampling times. For this reason data of these horses was not included in the statistical analysis. Descriptive data of these horses, including the different coagulation parameters studied and the degree of coagulopathy per diagnostic group can be found in **Table 2.1**.

Table 2.1. Progression over time of coagulation parameters and degree of coagulopathy in the different diagnostic groups of non-surviving colicky horses because of poor prognosis *(mean, IQR)*.

		D-dimer (ng/mL)	**PT** (sec)	**aPTT** (sec)	**AT** (%)	**NORMAL** % (n)	**ACT. COAG** % (n)	**SUBCL DIC** % (n)
OBSTRUCTIVE	*Admission*	**688** [250; 6,831]	**13.3** [12.3; 13.8]	**56.2** [49.1; 58.1]	**220** [212; 220]	**100** (4)	**0** (0)	**0** (0)
	Hospitalization	**4,243** [782; 5,000]	**14.6** [13.4; 16]	**59.8** [55.7; 71.2]	**140** [116; 144]	**40** (2)	**60** (3)	**0** (0)
N=6	*Antemortem*	**2,746** [2,662; 2,831]	**12.1** [11.7; 12.5]	**48** [48; 48]	**158** [142; 174]	**100** (2)	**0** (0)	**0** (0)
INFLAMMATORY	*Admission*	**2,771** [1,795; 3,913]	**15.9** [12.4; 21.6]	**61.3** [41.9; 87.7]	**214** [126; 256]	**40** (2)	**0** (0)	**60** (3)
	Hospitalization	**2,623** [1,539; 4,118]	**20.5** [16.5; 20.9]	**77.4** [65.7; 92.9]	**104** [84; 144]	**0** (0)	**0** (0)	**100** (3)
N=8	*Antemortem*	**2,989** [171; 8,616]	**18.4** [13.7; 25.4]	**80.6** [64.3; 94.8]	**93** [81; 104]	**25** (1)	**25** (1)	**50** (2)
ISCHEMIC	*Admission*	**2,122** [800; 4,439]	**13.3** [12.4; 14.4]	**56.8** [41; 60.4]	**201** [188; 208]	**82** (9)	**9** (1)	**9** (1)
	Hospitalization	**6,819** [2,890; 14,494]	**22.4** [17.8; 30.2]	**139.6** [104.9; 161.4]	**133** [104; 147]	**0** (0)	**0** (0)	**100** (7)
N=11	*Antemortem*	**1,454** [810; 3,136]	**25.4** [21.9; 30.3]	**126.6** [55; 174.5]	**78** 78 [78; 78]	**0** (0)	**0** (0)	**100** (2)
PERITONITIS	*Admission*	**4,405** [2,596; 5,000]	**13.9** [13.8; 14.4]	**49.4** [43.6; 59.7]	**180** [152; 194]	**80** (4)	**20** (1)	**0** (0)
	Hospitalization	**4,114** [3,352; 5,771]	**19** [14.3; 19.4]	**71.5** [62.9; 88.6]	**144** [136; 152]	**38** (3)	**0** (0)	**62** (5)
N=6	*Antemortem*	**10,000** [7,384; 18,028]	**13.4** [13.1; 14]	**60.7** [52,9; 64,1]	**98** [90; 150]	**60** (3)	**20** (1)	**20** (1)

Progression of coagulation parameters during hospitalization

D-dimers

Evolution of plasma D-dimer concentration during hospitalization in the different diagnostic groups is represented in **Figure 2.1**.

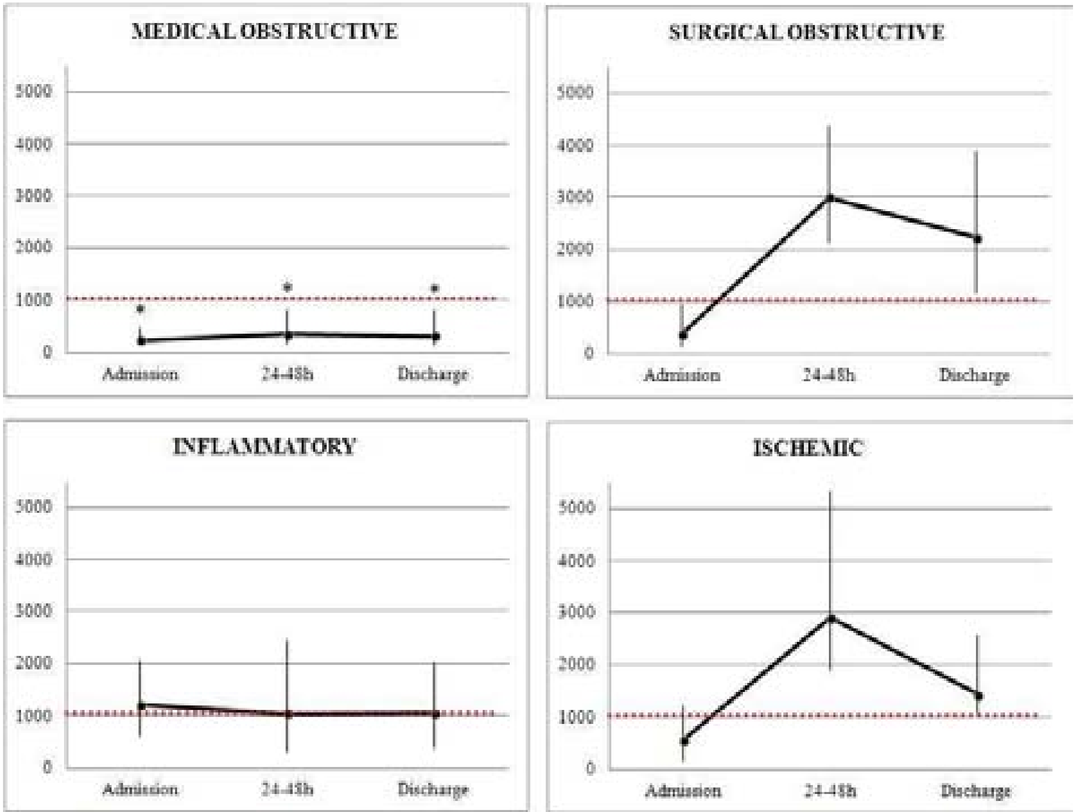


Figure 2.1. Progression of plasma D-dimer concentration during hospitalization in the different diagnostic groups of surviving horses *(mean, IQR).* Significant differences (P≤0.01) in regard to other groups are indicated (*). Dashed lines indicate plasma D-dimer concentration considered abnormal (*Monreal 2000*).

Values of D-dimers at 24-48h and discharge were significantly (P<0.001) different compared to admission values independently of diagnosis (time effect).

Furthermore, for each time point, D-dimer concentrations in medical obstructive cases were significantly lower ($P \leq 0.001$) than those in surgical obstructions, inflammatory or ischemic disorders (diagnosis effect). The presence of thrombophlebitis or the treatment with LMWH did not change the significance of the differences observed. When the different time points were considered by diagnosis, significant differences ($P<0.001$) were found at admission, 24-48h and discharge between medical obstructive group and the rest of diagnostic groups.

Prothrombin time

Values of PT at 24-48h and discharge were significantly ($P<0.001$) prolonged compared to admission values independently of diagnosis (**Table 2.2**). Furthermore, the progression over time of PT between medical obstructive and ischemic groups was significantly different ($P=0.02$). In the medical obstructive group they remained relatively stable during hospitalization and within normal values, while in the ischemic group PT values were already over the normal range on admission, there was a marked prolongation after surgery, but by the time of discharge values were practically normal. When results were adjusted for the presence of thrombophlebitis, significant differences were also found between ischemic and surgical obstructive groups ($P=0.037$) and between ischemic and inflammatory groups ($P=0.032$). LMWH therapy influenced PT values significantly ($P=0.018$), with treated animals having more prolonged values of PT, independently of diagnosis. When the different time points were considered, no significant differences were observed between diagnostic groups.

Activated partial thromboplastin time

Values of aPTT at 24-48h and discharge were significantly ($P \leq 0.015$) prolonged compared to admission values independently of diagnosis (**Table 2.2**). Furthermore, the progression over time of aPPT between medical obstructive and ischemic groups was significantly different ($P=0.041$). Mainly, ischemic colics had prolonged aPTT values on admission but, in contrast to medical obstructive cases, by the time of discharge values were practically normal. When results were adjusted for the presence of thrombophlebitis, significant differences were also found between ischemic and inflammatory groups ($P=0.012$). LMWH therapy influenced aPPT values significantly ($P=0.02$). When the different time points were considered by diagnosis, significant differences ($P<0.05$) were found on admission, 24-48h and discharge between medical obstructive and surgical obstructive groups and between medical obstructive and inflammatory groups. Despite at 24-48h aPTT was longer for surgical colics, by the time of discharge medical obstructions were the group with the highest values.

Antithrombin activity

Values of AT at 24-48h and discharge were significantly ($P<0.01$) lower compared to admission values independently of diagnosis (**Table 2.2**). The progression over time of AT was significantly different between medical obstructive and inflammatory or ischemic groups. Mainly stable in the first group, in the two last groups AT decreased markedly at 24-48h and recovered towards the end of hospitalization. Furthermore, surgical obstructive patients had a significantly different AT profile compared to ischemic group. Values on admission were lower in ischemic patients, despite subsequent evolution were very similar. When results were adjusted for the presence of thrombophlebitis, this last difference was not observed. Taking into account the treatment with LMWH, no difference was observed between medical obstructive and inflammatory groups. When the different time points were considered by diagnosis, AT was significantly lower (P<0.05) in ischemic group on admission and at 24-48h compared to medical and surgical obstructive groups, despite values remained always within the normal range.

Table 2.2. Progression over time of coagulation parameters during hospitalization in the different diagnostic groups of surviving horses *(mean, IQR)*.

		PT (sec)	**aPTT** (sec)	**AT** (%)
MED OBSTR	Admission	**12,1**	**55,6**	**211**
		[11,3-13,9]	*[44,5-65,7]*	*[196-222]*
	24-48h	**12,6**	**63,5**	**204**
		[11,9-14,4]	*[49,1-67,1]*	*[186-216]*
	Discharge	**12,7**	**57,8**	**196**
		[11,1-14,0]	*[46,0-72,2]*	*[180-218]*
SURG OBSTR	Admission	**12,0**	**48,4**	**220**
		[11,1-16,6]	*[44,8-59,1]*	*[196-246]*
	24-48h	**15,8**	**63,6**	**174**
		[13,1-18,1]	*[52,6-82,0]*	*[152-196]*
	Discharge	**11,3**	**46,0**	**204**
		[10,5-13,7]	*[42,7-57,1]*	*[189-222]*
INFLAMMAT	Admission	**12,9**	**54,4**	**203**
		[11,8-15,0]	*[48,2-59,4]*	*[188-216]*
	24-48h	**13,7**	**53,7**	**170**
		[12,7-15,4]	*[47,7-79,1]*	*[150-208]*
	Discharge	**11,0**	**47,4**	**190**
		[10,7-12,9]	*[41,9-53,2]*	*[166-206]*
ISCHEMIC	Admission	**13,9**	**61,2**	**197**
		[12,4-14,9]	*[49,8-67,3]*	*[178-210]*
	24-48h	**15,6**	**64,7**	**149**
		[13,9-19,0]	*[51,2-81,4]*	*[134-164]*
	Discharge	**12,3**	**51,4**	**186**
		[11,4-13,9]	*[45,8-63,6]*	*[170-206]*

Progression of coagulopathies during hospitalization

From admission to discharge, the percentage of horses with activation of coagulation system in the medical obstructive group increased from 39% to 50% (**Table 2.3**). Only one horse (4%) in this group was considered to be in subclinical DIC.

In the surgical obstructive group, half of horses had a normal coagulation profile on admission and the other half presented some kind of coagulopathy (mostly activation), whereas by 24-48h of hospitalization the percentage of coagulopathies increased (especially subclinical DIC) to 96%. By the time of discharge, only one horse (4%) remained in subclinical DIC, whereas 83% of patients presented still activation of coagulation.

Regarding evolution in the inflammatory group, almost three quarters of the patients presented some kind of coagulopathy already on admission. At 24-48 h, 41% of horses had normalized the coagulation profile, but the percentage of horses in subclinical DIC had increased to 14%. By discharge none of the horses was in subclinical DIC, despite 59% had still activation of coagulation.

On admission, already 59% of horses in the ischemic group presented activation of coagulation, despite none were in subclinical DIC. By 24-48 h, after surgery, 37% of horses were in subclinical DIC and only 11% presented a normal coagulation profile. By discharge, most of horses with coagulopathy had activation of coagulation and only one horse (5%) remained still in subclinical DIC.

Table 2.3. Progression over time of coagulopathies during hospitalization in the different diagnostic groups of surviving horses.

		NORMAL **%** (n)	ACTIV COAG **%** (n)	SUBCLIN DIC **%** (n)
MED OBSTR	*Admission*	**58** (15)	**39** (10)	**4** (1)
	24-48h	**42** (11)	**50** (13)	**8** (2)
	Discharge	**46** (12)	**50** (13)	**4** (1)
SURG OBSTR	*Admission*	**50** (12)	**46** (11)	**4** (1)
	24-48h	**4** (1)*****	**54** (13)*****	**42** (10)*****
	Discharge	**13** (3)*****	**83** (20)*****	**4** (1)*****
INFLAMMAT	*Admission*	**27** (6)	**64** (14)	**9** (2)
	24-48h	**41** (9)	**45,5** (10)	**14** (3)
	Discharge	**41** (9)	**59** (13)	**0** (0)
ISCHEMIC	*Admission*	**47** (9)	**53** (10)	**0** (0)
	24-48h	**11** (2)	**53** (10)	**37** (7)
	Discharge	**11** (2)	**84** (16)	**5** (1)

* Significantly different ($p \leq 0.01$) from medical obstructive cases

Hypercoagulation seen after abdominal surgery

While on admission there were no significant differences, at 24-48h (after surgery) the distribution of coagulopathies was significantly different in the surgical obstructive group compared to the medical obstructive one ($P<0.01$, **Table 2.3**). The proportion of horses with activation of coagulation and subclinical DIC was higher after surgery. These differences remained significant by the time of discharge ($P=0.018$).

Observing the distribution of individual coagulation parameters in medical and surgical obstructive groups, it could be noted that the percentage of horses with D-dimer concentration over 1000 ng/mL increased fourfold (from 21% (n=5) on admission to 92% (n=22)) after surgery (24-48h) in surgical obstructive cases, whereas just doubled (from 12 (n=3) to 23% (n=6)) in the medical obstructive group. Furthermore, while most horses (76%) with medical obstructive colic had plasmatic D-dimer concentrations below 1000 ng/mL during the whole hospitalization period, about 90% of horses with surgical colic presented D-dimer concentration above 1000 ng/mL after surgery, and this percentage remained high (80% (n=19)) by the time of discharge. The proportion of horses with abnormal values of PT (>15s) and aPTT (>63s) on admission and at 24-48h remained quite stable in medical cases (21% (n=5) and 25% (n=6) for PT and 33% (n=8) and 42% (n=10) for aPTT) whereas it was almost doubled in surgical ones (from 38% (n=9) to 61% (n=14) for PT and from 25% (n=6) to 52% (n=12) for aPTT).

Discussion #2

This study documents that plasma D-dimer concentration in hospitalized horses with colic is related to the type of gastrointestinal disorder and the time of hospitalization. The medical obstructive group has significantly lower plasmatic D-dimer concentrations throughout hospitalization, as well as a lower percentage of horses in subclinical DIC when compared to other groups. Interestingly, a marked hypercoagulability (increased percentage of horses in subclinical DIC) is also seen postoperatively in surgical obstructive and ischemic groups. Activation of coagulation was still present in these horses by the time of discharge.

In horses, determination of plasma D-dimer concentration is considered the most sensitive test to diagnose hypercoagulative states, thromboembolic disease and DIC (*Monreal 2003*). Marked increases on admission plasma D-dimer concentration in horses with colic have been associated to ischemic and inflammatory diagnoses, especially peritonitis, as well as to a poor prognosis (*Sandholm 1995, Cesarini 2009b, Delgado 2009*). In this study, and according with the literature, ischemic and inflammatory colics have significantly higher plasma D-dimer concentrations than medical obstructive colics at all time points. Interestingly, depending on diagnosis, at 24-48h of hospitalization plasma D-dimer concentrations can be very different when compared to admission values. This is especially true for both groups of surgical colics, where important increases in D-dimer concentration are observed postoperatively. Increased values of plasma D-dimers occur, among others, in situations of clinical or subclinical DIC (*Dallap 2003, Feige 2003a, Monreal 2003*). Interestingly the percentage of horses with different degrees of coagulopathy (including subclinical DIC) was also higher in surgical and inflammatory colics. The presence of thrombophlebitis or the treatment with LMWH did not change the differences observed in D-dimer concentration due to diagnosis.

It has been reported that plasma D-dimer concentrations return to reference values by day 6 of hospitalization in certain types of colic, whereas in others it remained significantly increased for a longer period (*Feige 2003a*). In the present study, around 85% of horses with medical obstructive colic had plasmatic D-dimer concentrations <1000 ng/mL during the whole hospitalization period. On the other hand, about 90% of horses with surgical colic presented D-dimer concentration >1000 ng/mL after surgery and this percentage remained high (80%) by the time of discharge.

Prasse et al (*Prasse 1993*) had similar findings and trends in the coagulation abnormalities of several groups of colic horses (prolonged clotting times and decreased AT activity), with alterations being more severe in horses with strangulating obstructions and, to a lesser extent, in horses with inflammatory disorders. Despite diagnostic groups not being the same as in the present study, they showed similar tendencies over time. Evaluating progression of coagulopathies during 10 days of hospitalization, Feige and collaborators (*Feige 2003a*) found that prolonged aPTT was the most common abnormality. Clotting times were already prolonged preoperatively in all colic patients and continued to increase after the first day of surgery, peaking at 48h and normalizing by the time of discharge. Results follow the same evolution in the present study, despite values on admission being lower. It's interesting to notice that, by the time of discharge, medical obstructions were the group with the highest values of clotting times. This could be due to the fact that was the only group that did not receive LMWH during hospitalization. In fact, the statistical models used have shown an influence of receiving antithrombotic therapy in the results observed for both clotting times.

The percentage of horses in subclinical DIC was lower in medical obstructive colics when compared to other diagnosis. By 24-48h of hospitalization the percentage of coagulopathies (activation of coagulation and subclinical DIC) increased in all diagnostic groups. According with findings in the literature, surgical and inflammatory colics had the highest proportion of horses in subclinical DIC (*Monreal 2000, Dolente 2002, Dallap 2003*). By the time of discharge, few horses remained in subclinical DIC in all groups, whereas a variable percentage of patients presented still activation of coagulation. This improvement in the severity of the coagulopathy can follow the resolution of the colic disease itself but is probably also influenced by the antithrombotic therapy that a significant number of horses with inflammatory and ischemic conditions received during hospitalization.

Results of this study show that the proportion of coagulopathies was higher after surgery than in medical colics. The percentage of patients in subclinical DIC increased tenfold after surgery in surgical obstructive cases, whereas just doubled in medical obstructive colics. Hemostatic changes after a colic surgery have been previously evaluated by determination of plasma D-dimer concentrations, and these were reported to rise during the initial days after the surgical event in these patients (*Moore 1995, Feige 2003ab*). Some studies consider that surgical trauma has a minor influence on plasma D-dimer concentration compared with the gastrointestinal disorder itself (*Feige 2003a*). In contrast, an experimental study (*Moore 1995*) suggested that ventral midline celiotomy, exteriorization and instrumentation of the large colon, induced the same degree of hemostatic activation as ischemia-reperfusion injury itself. That could explain the similar evolution of the hemostatic profile (elevation of D-dimer concentration, prolongation of PT and decrease of AT) and the increased number of patients in subclinical DIC postoperatively in both surgical groups in this study, regardless of the very different pathophysiology of obstructive and ischemic colic disease. Moreover, almost 90% of surgical colics (obstructions and ischemic) presented some kind of coagulopathy by the time of discharge.

Several limitations should be considered when interpreting data from this study. Colic patients constitute a heterogeneous population, and even animals with the same diagnosis may have different severity, may have been admitted at different stages of the disease or in some cases they may have concomitant factors that could affect hemostatic profile (i.e. thrombophlebitis). Only surviving animals were finally included in the study, due to the fact that most animals with poor outcome were dying/being euthanized during surgery or shortly after admission, and so there was not really follow up in those cases. Unfortunately, there were some missing values in the sampling population of this study. The clinical setting, with emergency admissions and different clinicians involved in sample collection, resulted in missing time points or insufficient sample to determine all the required hemostatic parameters in some cases. Despite using statistical models that try to account for this problem, the amount of missing values makes the actual population size of the study quite small. Finally, administration of antithrombotic therapy in the study population was not randomized, which makes difficult to draw conclusions about its use. Most horses in the ischemic and inflammatory group were treated with LMWH, which obviously influences coagulation parameters and makes comparison between groups difficult. As LMWH was administered following the clinician's criteria, it was frequently used in animals more severely affected, thought to be more at risk of coagulopathy. Some of the results obtained in this study are influenced by this fact, being animals treated with LMWH the ones with more prolonged clotting times, for example.

In conclusion, medical obstructive colics have less severe coagulopathies and lower plasmatic D-dimer concentrations throughout hospitalization compared to other diagnosis. Depending on diagnosis, at 24-48h of hospitalization the hemostatic profile can be very different when compared to admission values. Most horses with gastrointestinal inflammatory conditions presented some coagulopathy already on admission, which was milder at discharge. In contrast, both ischemic disorders and surgical obstructions developed a marked hypercoagulability postoperatively, still present when the patient was discharged from the hospital. These patients have a higher risk of DIC and thromboembolic disease and would eventually benefit from early antithrombotic therapy. Evaluation of plasma D-dimer concentration could be a useful tool to monitor these hypercoagulative states in equine colic cases.

Study#3

Association between necropsy evidence of disseminated intravascular coagulation and coagulation variables before death in horses with colic

Abstract #3

Background: Disseminated intravascular coagulation (DIC) is a frequent complication in horses with severe gastrointestinal disorders. Postmortem studies have found fibrin microthrombi in different tissues of these horses, but currently there are no studies relating these histopathological findings with data from the hemostatic profile obtained before death in such patients.

Hypothesis: The plasmatic levels of different hemostatic parameters and the severity of laboratorial coagulopathy are related to the presence and severity of fibrin deposits observed postmortem in horses with severe gastrointestinal disorders.

Animals: Tissue-organ samples collected during postmortem examinations on 48 horses with colic and results of antemortem hemostatic profile from the same horses.

Methods: Tissue samples (kidney, lung and liver) were stained with phosphotungstic acid hematoxylin (PTAH) and immunohistochemical (IHC) methods for blinded histologic examination. A fibrin score (grades 0 to 4) was established for each tissue sample and for each horse for both techniques, as well as the presence of histopathologic DIC. Blood samples were collected between 0 and 24h before death/euthanasia and plasma D-dimer concentration, clotting times and antithrombin activity were determined, as well as the presence of laboratorial DIC. Both histological and laboratorial data from the same horses were retrospectively compared.

Results: No association was found between antemortem laboratorial classification of DIC and postmortem classification based on tissue fibrin deposition. None of the four hemostatic parameters measured was significantly different between horses with or without histopathologic DIC, based on PTAH or IHC findings. Furthermore, no significant differences were detected when horses with tissue scores ≥ 2 were compared to those with lower scores or when cut-off values for each parameter were used to classify the population.

Conclusion and clinical importance: Abnormalities of the routine clotting profile, including plasmatic D-dimer concentration, do not seem to be useful in predicting those cases in which histological evidence of disseminated intravascular coagulation will be found at necropsy.

Introduction #3

Coagulopathies and disseminated intravascular coagulation (DIC) are a frequent finding in horses with gastrointestinal disorders, especially in those with poor prognosis (*Johnstone 1986ab, Prasse 1993, Monreal 2000, Dolente 2002, Dallap 2003, Cesarini 2010*). Confirmation of DIC is challenging because it is considered a complex dynamic syndrome and patients may be examined at any point of the continuum (*Stokol 2010*).

Because disseminated intravascular coagulation is such a dynamic process, clinical signs and results of coagulation tests may continually change. A single set of results should therefore be interpreted with caution and sequential testing is considered much more informative (*Wilde 1988, Cesarini 2009*). Individual laboratory tests have variable sensitivity and specificity for this syndrome and no single laboratory test can be used to make a definite diagnosis of DIC (*Wilde 1988, Welch 1992, Stokol 2010*). Traditional criteria for the diagnosis of overt DIC in horses include a combination of three or more abnormal results from a set of specific hemostatic parameters (*Welch 1992, Monreal 2000, Dallap 2003, Cesarini 2009*).

Histopathologic diagnosis of DIC is based on the detection fibrin thrombi throughout the microvasculature of various organs (*Hayashi 1989*), and it is considered a “gold standard” test for the confirmation of DIC in veterinary medicine (*Stokol 2010*). The pathological diagnosis of disseminated intravascular coagulation is based primarily on the finding of fibrin microthrombi in arterioles, capillaries, and venules in the absence of any local disease that may have accounted for their deposition (*Tanaka 1983, Kojima 1983*). In human medicine, detection of microvascular fibrin deposition in one or more organs is considered essential for histopathologic diagnosis of DIC (*Robboy 1972, Kim 1976, Wilde 1988*), and the same is suggested in animals (*Bateman 1999*). Several studies by Cotovio and collaborators have reported the occurrence of fibrin deposits in tissues of septic foals and horses with severe gastrointestinal disorders (*Cotovio 2007ab& 2008*). In non-surviving horses with colic, a high percentage of cases had fibrin microthrombi in different tissues, and the lung was the organ most commonly affected. This marked fibrin deposition in horses was consistent with capillary microthrombosis, multiorgan failure syndrome, and DIC (*Cotovio 2007b*). Results from these studies suggested that both phosphotungstic acid hematoxylin (PTAH) and immunohistochemical (IHC) staining methods were reliable in demonstrating fibrin deposits in the capillary meshwork of horses at risk of DIC, despite IHC was considered to be slightly more sensitive (*Cotovio 2007b*).

Current trends in human medicine emphasize the need for early recognition of DIC, allowing then precocious treatment and hopefully improving prognosis in patients at risk of uncompensated coagulopathy (*Stokol 2010, Monreal 2008*). Clarifying the possible relationship between the antemortem clinical and laboratory diagnosis of DIC with its definitive postmortem confirmation could help to identify those cases with increased risk of organ failure. In human medicine, abnormalities in the routine coagulation profile were not found to be useful in predicting those cases with postmortem histological evidence of DIC (*Wilde 1988*). Interestingly, the same authors suggest that additional tests that specifically detect lytic products of deposited crosslinked fibrin, such as D-dimer determination, may be more accurate in identifying those patients who have evidence of DIC at necropsy. To the author’s knowledge, there are currently no studies in horses that have analyzed coagulation data before death to determine whether any differences exist between patients with or without evidence of disseminated intravascular coagulation at necropsy.

Considering that DIC is common in horses with severe colic and that marked fibrin deposition in these patients is consistent with microthrombosis and organ failure, the hypothesis of the present study was that the plasmatic levels of different hemostatic parameters, including D-dimers, and the severity of laboratorial coagulopathy would be related to the presence and severity of fibrin deposits observed postmortem in horses with severe gastrointestinal disorders.

Thus, the main objectives of this study were: 1) to determine the association between the antemortem hemostatic profile, including D-dimer concentration, and the presence of fibrin deposition in different postmortem tissues of horses with gastrointestinal disorders; and 2) to evaluate the association between the laboratorial diagnosis of DIC and the presence of histopathologic evidence of DIC. Secondarily, the possible influence of diagnosis in the previous findings was studied.

Materials and Methods #3

Animals

Tissues and blood samples were obtained from 48 horses with colic that died a few hours after admission to the “Fundació Hospital Clínic Veterinari de la Universitat Autònoma de Barcelona”, or that were euthanized because of poor prognosis or financial constraints. Horses with colic and an additional clinical problem (i.e. pneumonia) were excluded. Most tissue samples and some of the coagulation profile results used in this study had also been included in previous studies (*Cotovio 2007b, Cesarini 2009& 2010*).

Horses included in this study were classified into four groups according to their final diagnoses, based on clinical and postmortem examination findings: *peritonitis group* (n=19), *ischemic group* (n=16), *enteritis group* (n=4) and *obstructive group* (n=9). The reader is referred to the initial section on “Common Aspects of Materials and Methods” (pages 41-44) for details about inclusion criteria of this classification. Due to the small number of horses in each of the two last groups (enteritis and obstructive), for statistical purposes these were grouped together as “Other diagnoses”.

All horses with peritonitis were euthanized within hours after admission because of a poor prognosis as soon as the clinical diagnosis was made. Horses in the ischemic group were euthanized because of poor prognosis or economical restraints shortly after admission or during laparotomy. Horses with enteritis or obstructive problems did not respond to medical therapy, showed increasing abdominal pain and/or distension, and were euthanized because of poor prognosis or economic limitations.

Sampling and Processing of Tissues

Tissue samples from kidneys, lungs, and liver were taken during laparotomy or at necropsy (1–48 hours after death) for further histopathologic study. The renal tissue specimens were taken with special care to include cortex and medulla. The pulmonary samples were obtained from the caudal lung lobe, and the hepatic samples were taken randomly from no specific area of the liver. Samples were fixed in 10% neutral formalin, processed in an automated tissue processor and embedded in paraffin. Three-millimeter paraffin sections were cut with a microtome and routinely stained with PTAH by standard procedures (*Wilson 2002*). For IHC, 3-mm sections were mounted on silane-coated slides and dried. Immunolabeling with polyclonal rabbit anti-human fibrinogen/fibrin antibody was performed with a streptavidin biotin complex method as described elsewhere (*Cotovio 2007b*).

The presence of microvascular fibrin deposits in glomerular and alveolar capillaries and in hepatic sinusoids was recorded separately for each sample stained with PTAH and IHC, and extravascular fibrin deposits were not considered to avoid possible misclassification of fibrin of inflammatory origin.

Regarding the PTAH-stained slides, the amount of fibrin present in kidney, lung and liver sections was graded from 0 to 4 as described previously (*Gómez 1989, Montes 2002, Cotovio 2007ab& 2008*). Briefly, 0 was absence of fibrin; 1, presence of partial fibrin deposits in some glomerular capillaries; 2, presence of partial deposits in all glomerular capillaries; 3, presence of large quantities of fibrin in all glomeruli; and 4, presence of fibrin thrombi in glomerular capillaries and in noncapillary vessels. For each renal section 10 fields were examined, and the average value from all of them was calculated. For lung and liver sections 20 fields were examined, and the number of fields positive for the presence of intravascular fibrin in alveolar capillaries and hepatic sinusoids was counted, respectively. Sections where 20 of 20 fields were positive received a score of 4; sections where none of the fields were positive received a 0 score.

For grading the amount of fibrin deposits in the three tissues when using the IHC method, slides were assigned a fibrin score (from 0 to 4) as previously described (*Cotovio 2007b*); briefly, 0 was absent staining or positive in <10% of the glomeruli; 1, staining in glomerular capillaries in 11–25% of glomeruli in nonconsecutive fields; 2, staining in glomerular capillaries in 26–50% of glomeruli; 3, staining in glomerular capillaries in 51–75% of glomeruli; and 4, staining in glomerular capillaries in >75% of glomeruli. A 0–4 scoring system also was designed for lung and liver sections: 0, absent staining or <10% positive fields; 1, 11–25% positive fields with staining in alveolar capillaries or hepatic sinusoids in nonconsecutive fields; 2, 26–50% positive fields with staining in alveolar capillaries or hepatic sinusoids in half of the fields in an almost continuous staining pattern; 3, 51–75% positive fields with staining in alveolar capillaries or hepatic sinusoids in >50% of the fields in a continuous staining pattern; and 4, >76% positive fields with staining in alveolar capillaries or hepatic sinusoids in a continuous staining pattern with a fibrillar meshwork.

To summarize all of the information about fibrin deposition in tissues, each horse was assigned a score (0 to 4), depending on the amount of fibrin thrombi observed in kidney, lung and liver. Briefly, a horse was classified as grade 4 when it had at least 1 tissue sample with a 4 score; grade 3 horses were those with at least 1 tissue sample scored 3 and no samples with higher scores; horses classified as grade 2 were those that had at least 1 tissue sample scored 2 and no tissue samples with higher scores; and grade 1 horses were those that had at least 2 tissue samples scored 1 and no tissue samples of higher score. The rest of the horses were classified as grade 0.

For the purpose of this study and based on similar human criteria (*Robboy 1972, Kim 1976, Kojima 1983, Tanaka 1983, Wilde 1988*), a horse was considered to have histopathologic DIC when any degree of microvascular fibrin deposition was detected in two or more of the studied organs.

Blood Sampling and Measured Parameters

Citrated blood samples were obtained by jugular venipuncture between 0 and 24 hours before death or euthanasia. They were centrifuged and plasma was frozen at –80ºC until analysis. Plasma D-dimer concentration, PT, aPTT, and AT were determined in duplicate for all samples. The reader is referred to the section on “Common Aspects of Materials and Methods” (pages 41-44) for further details about the techniques of blood sampling and processing.

According to the number of abnormal hemostatic parameters, horses were classified as having a *normal hemostatic profile* (0 abnormal parameters), having *activation of the coagulation* (1 or 2 abnormal parameters) system or being in *laboratorial* (*subclinical) DIC* (3 or 4 abnormal parameters). Further details about this classification are described in the previous section “Common Aspects of Materials and Methods” (pages 41-44).

Statistical Analysis

Results were expressed as frequencies and percentages for qualitative variables. For quantitative variables, median was used as central estimation of values and interquartile range (percentiles 25th and 75th) were used as measurement of precision of results. The relationship between the analytical results and the evaluation of histopathology was assessed by estimating odds ratios (OR) and confidence intervals at 95% from logistic regression models. Cut-off values used in the diagnosis of DIC (*Monreal 2000, Armengou 2008, Cesarini 2009& 2010*) for each of the measured parameters were also tested in logistic regression models. An additional cut-off value of >4000 ng/mL, previously shown to be associated with outcome (*Cesarini 2010*), was used for plasmatic D-dimer concentrations. Based on previous studies (*Cotovio 2008*) and considering that a mild fibrin deposition (grade 1) may not be sufficient to cause organ dysfunction, a minimum cut-point of 2 for fibrin deposits was established classify the study population in order to evaluate the risk of organ failure. For the assessment of the influence of diagnosis in the evaluation of analytical results adjusted ORs were calculated. All analyses were performed using the statistical soft ware package SPSS ver. 20 (Chicago, IL, USA) and a type I error of 5% was considered in all statistical analyses.

Results #3

Animals

The gender distribution of the 48 horses was 16 females (33%), 14 intact males (29%) and 15 geldings (31%). No gender information was available for 3 horses. The mean age (standard deviation) of the population was 9.7 years (±7 years). Twenty (42%) horses were Andalusians, 7 (15%) crossbred, 4 (8%) Westfalians, 3 (6%) Friesians, and the other 8 (17 %) were a mix of other breeds. No breed information was available for 6 horses. Four horses (8 %) died due to severity of the primary gastrointestinal disease or its complications. The other 44 horses (92 %) were euthanized, 38 (86%) due to poor prognosis of their condition at the time of death and 6 (14%) because financial limitations did not allow adequate treatment (i.e. surgery).

Association between hemostatic profile and fibrin deposition in different tissues

None of the four hemostatic parameters measured (D-dimer, PT, aPTT, AT) was significantly different between horses that had or not fibrin deposits in kidney, lung or liver detected by any of both methods (PTAH and IHC) used in this study. No significant differences were detected when chosen cut-off values for each parameter were used to classify the population.

Results of the association between the hemostatic profile and histopathologic DIC are shown in **Table 3.1**. None of the four hemostatic parameters measured was significantly different between horses that were

Table 3.1. Results of logistic regression models including hemostatic parameters and postmortem histopathologic data for each of the two methods used to detect fibrin deposition in tissues.

	Tissue PTAH staining						Tissue IHC technique					
	Histopathologic DIC		*P value*	Tissue score		*P value*	Histopathologic DIC		*P value*	Tissue score		*P value*
Laboratory data	**YES**	**NO**		**≥ 2**	**< 2**		**YES**	**NO**		**≥ 2**	**< 2**	
D-dimer (ng/ml)	3,378 [742; 5,000]	2,603 [1,054; 4,418]	0.829	2,739 [742; 4,418]	2,627 [1,145; 4,745]	0.870	2,777 [1,235; 4,418]	2,728 [742; 4,928]	0.741	2,219 [1,026; 4,418]	2,829 [943; 4,745]	0.942
PT (sec)	13.8 [13.1; 14.8]	14.9 [13.1; 19.8]	0.384	14.4 [13.4; 15.6]	15.1 [13; 19.8]	0.331	14.3 [13.8; 15.2]	14.5 [13.1; 17]	0.579	14.4 [11.7; 17]	14.7 [13.2; 17.5]	0.580
aPTT (sec)	59 [51; 63]	60 [52; 68]	0.487	61 [51; 68]	55 [52; 85]	0.235	52 [43; 62]	60 [54; 68]	0.109	57 [45; 68]	60 [55; 68]	0.523
AT (%)	179 [149; 197]	163 [140; 194]	0.655	166 [146; 190]	174 [136; 198]	0.742	141 [116; 168]	177 [144; 198]	0.109	165 [144; 197]	167 [140; 194]	0.735

(PT, prothrombin time; PTTa, partial activated thromboplastin time; AT, antithrombin III; DIC, disseminated intravascular coagulation; PTAH, phosphotungstic acid hematoxylin stain; IHC, immunohistochemistry)

classified as having histopathologic DIC and those that were not. Similarly, no significant differences were detected when horses with tissue scores ≥ 2 were compared to those with lower scores or when cut-off values for each parameter were used to classify the population.

Association between histopathologic DIC and laboratorial DIC

From the 48 horses studied, following the classification based on results of the PTAH staining for fibrin deposition, 9 horses were in histopathologic DIC (microthrombi in 2 or more organs) and 39 horses were not presenting histopathologic DIC. Based on results of the IHC method for detecting fibrin deposition, 7 horses were in histopathologic DIC and 38 horses were classified as not presenting histopathologic DIC. Three horses could not be classified due to lack of histopathological data for at least one tissue.

Regarding laboratorial classification of coagulopathy, from the 48 horses, 5 were classified without coagulopathy, 25 as having activation of coagulation (1 or 2 hemostatic alterations) and 11 were considered to be in subclinical DIC. Seven horses could not be classified due to lack of hemostatic data for at least one parameter.

Results of the relation between laboratorial and histopathologic DIC classification are shown in **Table 3.2**. No association was found between antemortem laboratorial classification of DIC and postmortem classification based on tissue fibrin deposition. Furthermore, no significant differences were detected when horses with tissue scores ≥ 2 were compared to those with lower scores or when cut-off values for each parameter were used to classify the population.

Table 3.2. Results of logistic regression models including the different cut-offs tested for plasmatic D-dimer concentration, laboratory classification of DIC and its association with histopathologic classification of DIC, for each of the two methods to detect fibrin deposition in tissues.

	Tissue PTAH staining						Tissue IHC technique					
	Histopathologic DIC		*P value*	**Tissue score**		*P value*	**Histopathologic DIC**		*P value*	**Tissue score**		*P value*
Laboratory data	YES	NO		**≥ 2**	**< 2**		YES	NO		**≥ 2**	**< 2**	
D-dimer												
≤ 1000 ng/ml	33.3% (n=3)	21.1% (n=8)	0.439	26.3% (n=5)	21.4% (n=6)	0.698	0% (n=0)	29.7% (n=11)	1	20% (n=3)	25% (n=8)	0.706
> 1000 ng/ml	66.7% (n=6)	78.9% (n=30)		73.7% (n=14)	78.6% (n=22)		100% (n=7)	70.3% (n=26)		80% (n=12)	75% (n=24)	
≤ 4000 ng/ml	55.6% (n=5)	65.8% (n=25)	0.567	63.2% (n=12)	64.3% (n=18)	0.937	57.1% (n=4)	64.9% (n=24)	0.698	60% (n=9)	65.6% (n=21)	0.709
> 4000 ng/ml	44.4% (n=4)	34.2% (n=13)		36.8% (n=7)	35.7% (n=10)		42.9% (n=3)	35.1% (n=13)		40% (n=6)	34.4% (n=11)	
Laboratory DIC												
Normal	33.3% (n=3)	6.3% (n=2)	0.162	17.6% (n=3)	8.3% (n=2)	0.441	0% (n=0)	15.6% (n=5)	0.784	13.3% (n=2)	10.4% (n=3)	0.327
Activation	44.4% (n=4)	65.6% (n=21)		58.8% (n=10)	62.5% (n=15)		83.3% (n=5)	59.4% (n=19)		53.3% (n=8)	58.6% (n=17)	
Subclinical DIC	22.2% (n=2)	28.1% (n=9)		23.5% (n=4)	29.2% (n=7)		16.7% (n=1)	25% (n=8)		13.3% (n=2)	31% (n=9)	

(DIC, disseminated intravascular coagulation; PTAH, phosphotungstic acid hematoxylin stain; IHC, immunohistochemistry)

Influence of diagnosis

No differences in any of the previous associations were detected when the logistic regression models were adjusted for diagnosis.

Discussion #3

This study could not find significant differences in the plasmatic concentrations of any of the individual coagulation variables measured between those patients with and without evidence of disseminated intravascular coagulation at necropsy. Furthermore, considering the widely accepted traditional criteria for laboratorial diagnosis of overt DIC in horses (three or more abnormal results of a selection of hemostatic variables), no association was found between horses classified antemortem as being in subclinical DIC and those with histopathologic evidence of fibrin thrombi in 2 or more organs.

Plasmatic levels of D-dimers, PT, aPTT and AT and the severity of laboratorial coagulopathy were not related to the presence and severity of fibrin deposits observed postmortem, neither by PTAH stain nor by IHC methods. This is in agreement with results of similar studies in human medicine (*Wilde 1988*). Wilde et al couldn't find any differences either in the prevalence or magnitude of abnormality of any individual coagulation variable (PT, aPTT, thrombin time, fibrinogen, FDPs and platelet count) between intensive care unit patients with and without evidence of disseminated intravascular coagulation at necropsy, using the same criteria for histopathologic DIC that the present study (*Wilde 1988*). Furthermore, trying to find useful variables to predict DIC in patients with hemostatic abnormalities, another study concluded that the presence of pulmonary microthromboemboli was not significantly associated to clinical diagnosis of DIC (*Katsumura 1999*).

It has been suggested that specific markers of degradation of cross-linked fibrin, such as D-dimer concentration, could improve antemortem detection of those patients with postmortem evidence of disseminated intravascular coagulation (*Wilde 1988*). Nevertheless, based on results of the present study, D-dimer concentration was not found to be more useful than the rest of measured hemostatic parameters to detect patients with fibrin microthrombi in two or more organs at necropsy. Katsumura et al found that D-dimer levels were elevated not only in patients with pulmonary microthromboemboli but also in non-embolic patients (*Katsumura 1999*).

Horses considered to be in subclinical DIC antemortem were not classified more often as having histopathologic DIC, based on postmortem tissue fibrin deposition scoring. This could be due to misclassification of horses based on inaccurate laboratorial or histopathologic criteria. The laboratorial criteria used in this study are the classical criteria for diagnosing overt DIC in veterinary medicine, considering DIC when three or more abnormalities were detected in a selection of hemostatic parameters (*Stokol 2010*). In the present study, from 11 horses considered to have subclinical DIC based on laboratorial criteria, 5 did not present any microthrombi in any of the tissues collected when examined by PTAH and 3 when examined by IHC. These findings would suggest that abnormalities of the coagulation profile usually taken to indicate the presence of disseminated intravascular coagulation may not be specific for this condition.

In regard to histopathologic classification, there is no general agreement about the number of thrombi and the number of affected organs necessary to diagnose DIC but criteria used in this study have already been used in similar studies in human medicine (*Robboy 1972, Kim 1976, Kojima 1983, Tanaka 1983, Wilde 1988*). Furthermore, variables such as antemortem treatment, time from the occurrence of DIC to death and time from death to autopsy are known to affect thrombus lysis in human medicine (*Kaufman 1984*). In the present study, most patients were euthanized based on the clinical situation on admission, before receiving any more treatment than intravenous fluids and sedatives. Furthermore, to ensure that blood results were as representative as possible of the situation found at necropsy, we chose only horses in which the last blood sample available had been obtained less than 24h before death (less than 12h in 44 of the 48 horses). Other factors, such as postmortem fibrinolysis, amount of histological material sampled, staining techniques used and even missing microthrombi despite accurate microscopic examination, have created problems interpreting autopsy material in human medicine and may have contributed to failure of diagnosing postmortem DIC in some horses of this study (*Kaufman 1984*). Because thrombi may no longer be detectable if tissue fixation is not rapidly performed, postmortem examination in humans is not considered a definitive diagnostic tool for this syndrome (*Wilde 1988*).

The results of this study should be interpreted taking into account some limitations. The size of the studied population was reduced and mainly determined by the necessity of matching laboratorial and histopathologic data retrospectively from the same horses. Selecting animals to minimize the time between blood results and death (≤ 24h) and necropsy reduced even more the number of suitable patients.

In conclusion, in horses with gastrointestinal disorders, abnormalities of the routinely measured coagulation variables (D-dimer, PT, aPTT, AT) do not seem to be useful in predicting those cases in which histological evidence of disseminated intravascular coagulation will be found at necropsy. Based on the results of this study, antemortem plasmatic D-dimer concentration in horses with colic is not associated to the presence of postmortem fibrin thrombi in lung, kidney or liver.

Discussion

Discussion

Gastrointestinal problems are by far the most frequent disorder we encounter in our equine hospital. This thesis was born with the idea to help improve early detection of coagulopathies (specifically disseminated intravascular coagulation) in horses with colic, in order to implement early adequate treatment and improve the prognosis of affected patients. The study of a hospital population, even having some limitations, has the advantage of making possible a follow up of the disorder under investigation. Furthermore, in contrast to experimental studies, it represents the actual situation that the clinician will find when dealing with the disorder. To take advantage of these features and conduct a relatively complete investigation, it was decided to study a hospital population of colic horses at different points in the disease process. This approach seemed particularly suitable with disseminated intravascular coagulation being a dynamic syndrome. Multiple issues deserved to be studied:

- *Which hemostatic profile should we expect in the different diagnoses at the time of admission? Would it be useful for diagnostic purposes? Could we improve outcome prediction using D-dimer determination?*
- *How does this situation progress during hospitalization in the different diagnoses? Should patients with certain diagnoses receive specific hemostatic management to avoid further complications?*
- *Is it possible to predict accurately antemortem the presence of subclinical DIC? Does the hemostatic profile help to identify those animals with severe fibrin deposition and higher risk of organ failure?*

Based on these issues, and taking into account the limitations and routines of our hospital population, the three studies that form the core of this thesis were designed and conducted. In the following discussion the answers to the previous questions will be addressed.

What do we further know about coagulopathies in horses with colic?

This thesis contributes to provide new information on coagulopathies in horses with colic and in some aspects also confirms previous published findings about the subject.

Results of Study#1 confirm the fact that **coagulopathies are a frequent finding** in horses with gastrointestinal disorders, showing that between 31 and 87% (depending of the diagnostic group) of horses presented for colic at our hospital had an activated coagulation system **on admission**. Furthermore, up to 10 % of horses with diagnoses of enteritis or peritonitis were already in subclinical DIC. This is not surprising considering that both are severe inflammatory conditions and traditionally have been more frequently associated to DIC (*Monreal 2000, Dolente 2002, Dallap 2003, Delgado 2009*). These findings indicate that horses diagnosed with enteritis or peritonitis that remain hospitalized deserve a prompt management of their hemostatic imbalances to avoid worsening of the condition. Other diagnoses (such as ischemic disorders) would need to be retested after presentation or by use of a larger coagulation profile to better assess the associated coagulopathy.

The presence of coagulopathy on admission should also be taken into account when **formulating a prognosis** for the patient's condition. In the population considered in Study#1 horses had 8.6 times more probability to die if they were in subclinical DIC (>3 abnormal hemostatic parameters), and 5.3 times more probability if they presented an activated coagulation system (1 or 2 abnormal hemostatic parameters). These finding are in accordance as well with previous literature reports (*Johnstone 1986a*, *Prasse 1993, Collatos 1995a*, *Dallap 2003*).

Throughout hospitalization (Study#2) the evolution of the hemostatic profile seems to depend on diagnosis. Medical obstructive colics (impactions, displacements) had significantly lower percentage of horses in subclinical DIC whereas surgical and inflammatory colics had the highest proportion. These findings are in agreement with previous publications (*Prasse 1994, Monreal 2000, Dolente 2002, Dallap 2003).* Interestingly, by 24-48h of hospitalization the percentage of coagulopathies (activation of coagulation and subclinical DIC) increased in all diagnostic groups. For that reason, it would be interesting to perform control samples during hospitalization, and especially in the first 24-48 hours after admission, in horses with altered hemostatic parameters on admission or diagnosis "at risk". Nevertheless, alterations of the coagulation profile in horses that survived tended to normalize during hospitalization regardless of diagnosis, as observed in previous studies (*Feige 2003b*). This improvement in the severity of the coagulopathy can follow the resolution of the colic disease itself but is probably also influenced by the antithrombotic therapy that a significant number of horses with inflammatory and ischemic conditions received during hospitalization. Conclusions of this second study would certainly have been more useful for equine clinical practice if randomized LMWH therapy would have been used and data from dead horses included. Unfortunately, as already developed in the discussion of Study#2, limitations of the population studied and available data did not make it possible.

Interestingly, independently of diagnosis (obstructions or ischemic) all horses undergoing abdominal laparotomy developed a marked **postoperative hypercoagulability**. A previous study (*Feige 2003a*) concluded that it was the colic disease itself rather than the surgical procedure which was responsible for the coagulopathy observed in these patients but they were using a minor musculoskeletal surgery (Forssell's procedure) as a control surgery. Our results seem to support another publication that observed that healthy control horses undergoing an exploratory laparotomy also developed hemostatic alterations (*Moore 1995*). Based on results of Study#2, almost 90% of surgical patients presented some kind of coagulopathy by the time of discharge. These patients have a higher risk of DIC and thromboembolic disease and would eventually benefit from early antithrombotic therapy.

Finally, the third study of this thesis brings new information about interpretation of **antemortem hemostatic alterations**. Considering the widely accepted traditional criteria for laboratorial diagnosis of overt DIC in horses (three or more abnormal results of a selection of hemostatic variables), no association was found between horses classified antemortem as being in subclinical DIC and those with histopathologic evidence of fibrin thrombi in 2 or more organs. As already observed in human medicine (*Wilde 1988*), there seems to be no correlation between the antemortem (clinicopathological) diagnosis of DIC, defined as 3 or more laboratory hemostatic alterations, and the histopathological diagnosis of DIC, based on finding fibrin deposits in the microvasculature of postmortem tissues. Based on these results, it does not seem possible from a certain hemostatic profile in the living horse, to accurately predict the presence of fibrin deposition in kidney, lung or liver, and possibly a higher failure risk of these organs.

Potential clinical applications of measuring plasma D-dimer concentration in horses with colic

One of the main purposes of this thesis was to study the potential usefulness of determining plasma D-dimer concentration in horses with colic at different stages of the disease. All three studies bring new information about the relation of plasmatic D-dimers with diagnosis, prognosis and postmortem findings in horses with gastrointestinal disorders.

Results of Study#1 suggest that measurement of plasma D-dimer concentration **at the time of admission** can be used to facilitate diagnosis and outcome prediction in horses with colic. D-dimer concentration was the most sensitive coagulation parameter for these diagnostic and prognostic purposes of the four parameters tested. That confirms literature findings regarding the usefulness of plasma D-dimer concentration as a good prognostic aid in horses with colic (*Sandholm 1995*, *Armengou 2005*). Furthermore, this study brings new clinically useful information, proposing **several cut-offs with prognostic and diagnostic utility**. A potential cut-off value for nonsurvival was found at approximately 4,000 ng/mL and horses with levels over that value were found to be 8.78 times more likely to die. Complementary results suggest that for each horse with D-dimers above this cut-off that survives there are 6 that die (LR+ 5.9).

Nevertheless, some factors should be taken into account when interpreting increased D-dimer concentration to predict outcome, because they increase relatively quickly in DIC, but also in deep vein thrombosis or severe inflammation (*Dallap 2009*). D-dimers are considered a quite sensitive test but not extremely specific and a negative test result is much more helpful than a positive test result (*Dallap 2009*). In Study#1, 74.2% of survivors had plasma D-dimer concentrations within the normal range (<1,000 ng/mL) compared with 35% of nonsurvivors. Therefore, is paramount not to base prognosis estimation for the colic patient in a single parameter, but taking into account multiple factors known to be related with outcome, such as final diagnosis, cardiovascular parameters or blood lactate (*Dukti 2009, Southwood 2009*). Furthermore, some authors have found that the usefulness of D-dimers to predict outcome is somewhat dependent on the presenting complaint (i.e. in certain diseases all patients had increased levels and the concentration was not helpful in predicting survival (*Dallap 2009*). Interestingly, in human medicine it has been observed that the presence of DIC does not affect the outcome of patients with certain diagnoses (*Kawasugi 2011*).

Regarding **diagnostic usefulness of admission plasma D-dimer concentration**, some diagnoses were found to be associated with higher levels (enteritis and peritonitis) or specific cut-offs (medical *vs* surgical obstructive colics, ischemic problems requiring *vs* not requiring resection). Nevertheless, despite significant differences were detected in these cases, overlapping of values was common. Consequently, admission D-dimer concentration could be taken as complementary information to help differentiating horses with enteritis and peritonitis from other diagnoses, and especially to detect horses with poor prognosis, but diagnosis should still be based in results of major colic diagnostic tools, such as rectal examination, nasogastric intubation, abdominal ultrasound and peritoneal fluid analysis.

During hospitalization, evaluation of plasma D-dimer concentration could be a useful tool to monitor hypercoagulative states in equine colic cases. Results of Study#2 indicate that it is similarly related to the type of gastrointestinal disorder and the time of hospitalization. Depending on diagnosis, at 24-48h of hospitalization plasma D-dimer concentrations can be very different when compared to admission values. According to the literature (*Monreal 2000, Dolente 2002, Dallap 2003*), ischemic and inflammatory colics in this study had significantly higher plasma D-dimer concentrations than medical obstructive colics at all time points. Several cut-offs may have diagnostic utility in clinical settings, because around 85% of horses with medical obstructive colic had plasmatic D-dimer concentrations <1000 ng/mL during the whole hospitalization period, whereas about 90% of horses with surgical colic presented D-dimer concentration >1000 ng/mL after surgery and this percentage remained high (80%) by the time of discharge.

Important **increases in D-dimer concentration were observed postoperatively**, as mentioned in previous reports (*Moore 1995, Feige 2003ab*). Despite some studies consider that surgical trauma has a minor influence on plasma D-dimer concentration compared with the gastrointestinal disorder itself (*Feige 2003a*), results of Study#2 seem to support the fact that the abdominal laparotomy induces probably a significant hemostatic activation. That could explain why postoperatively surgical obstructive colics had a similar evolution of the hemostatic profile and increased coagulopathies as horses with ischemic disease. In dogs, plasmatic D-dimer concentration are reported to increase after routine ovario-hysterectomy (*Sobiech 2011*, *Moldal 2012*) but not after an experimentally induced moderate subcutaneous inflammation (*Bauer 2012*).

Based on results of Study#3, **antemortem D-dimer concentration** was not found to be more useful than the rest of measured hemostatic parameters to predict which patients presented fibrin microthrombi consistent with DIC at necropsy. Studies in human medicine could not find a relation between hemostatic profile and postmortem fibrin deposition in critical patients, but it was suggested that specific fibrinolytic markers could improve antemortem detection of these lesions (*Wilde 1988*). However, other authors found elevated D-dimers in both patients with and without thrombi (*Katsumura 1999*).

Overall, the three studies within this thesis have contributed to a better knowledge of coagulopathies in horses with gastrointestinal disorders and have provided new information about applications of D-dimer determination at different stages of the colic disease.

Conclusions

Conclusions

1. When determined on admission, D-dimer concentrations >4,000 ng/mL had the best likelihood ratio of death (LR+ 5.9, OR 8.8 (95% CI, 4.5–17.1, P< 0.001)) and could be useful as a cut-off to orientate prognosis in horses with gastrointestinal disorders.
2. Plasma D-dimer concentration measured on admission can be used to facilitate diagnosis and outcome prediction in horses with colic. Nonsurvivors had significantly ($P < 0.001$) higher plasma D-dimer concentrations at presentation than did survivors.
3. Depending on diagnosis, at 24-48h of hospitalization the hemostatic profile of horses with colic can be very different when compared to admission values.
4. Both ischemic disorders and surgical obstructions, despite having a very different pathophysiology, developed a marked hypercoagulability postoperatively that was still present when the patient was discharged from the hospital.
5. Abnormalities of the routinely measured coagulation variables (D-dimer, PT, aPTT, AT) do not seem to be associated to the presence of fibrin thrombi in lung, kidney or liver in horses with colic.
6. No association was found between horses with colic classified antemortem as being in subclinical DIC and those with histopathologic evidence of fibrin thrombi in 2 or more organs.
7. The type of gastrointestinal disease does not seem to influence the relationship between the antemortem hemostatic profile and the postmortem fibrin deposition in kidney, lung and liver in horses with colic.

Summary

Summary

Disseminated intravascular coagulation (DIC) is a frequent complication in horses with severe gastrointestinal (GI) disorders that has been associated with poor outcome. It results in microvascular fibrin deposition and eventually organ dysfunction. Recent experimental studies have shown that rapid detection and treatment of subclinical coagulopathies reduce morbidity and mortality associated with DIC. Plasma D-dimer concentration is considered the most sensitive test to diagnose thromboembolic disease and DIC in human medicine, and it has been shown to be a good predictor of mortality as well. In horses, D-dimers have been found to be useful to detect hypercoagulation in horses with colic and septic foals and some studies advocate its use as prognostic aid in horses with acute GI problems.

The objectives of this PhD thesis were to go further into the study of coagulopathies in horses with colic to improve early detection and management of DIC as well as to evaluate the potential clinical applications of the measurement of plasma D-dimer concentration in horses with colic.

With that purpose, three studies were conducted in a hospital population of horses with different diagnoses of gastrointestinal disease, evaluating hemostatic profiles (including D-dimer, prothrombin time, activated partial thromboplastin time and antithrombin III) and the presence of laboratorial DIC at three different stages of the disease: on admission, during hospitalization and before dead. Furthermore, in the third study, this clinicopathological data was compared to histopathological data from the same horses evaluating microvascular fibrin deposition to detect any correlation.

Results of the first study suggest that measurement of plasma D-dimer concentration at the time of admission can be used to facilitate diagnosis and outcome prediction in horses with colic. Furthermore, this study brings new clinically useful information, proposing several cut-offs with prognostic and diagnostic utility. During hospitalization (second study), evaluation of plasma D-dimer concentration has shown to be a useful tool to monitor hypercoagulative states in equine colic cases. Important increases in D-dimer concentration were observed postoperatively as well, as mentioned in previous reports. Finally, the third study concluded that antemortem D-dimer concentration was not more useful than the rest of measured hemostatic parameters to predict which patients presented fibrin microthrombi consistent with DIC at necropsy.

Overall, the three studies within this thesis have contributed to a better knowledge of coagulopathies in horses with gastrointestinal disorders and have provided new information about applications of D-dimer determination at different stages of the colic disease.

Resumen

La coagulación intravascular diseminada (CID) es una complicación frecuente en caballos con problemas gastrointestinales (GI) graves que ha sido asociada con un mal pronóstico. La CID tiene como consecuencia el depósito microvascular de fibrina que puede acabar causando disfunción multiorgánica. Estudios experimentales recientes han demostrado que la detección y tratamiento precoces de las coagulopatías subclínicas permite reducir la morbilidad y mortalidad asociadas con la CID. La concentración plasmática de dímero-D se considera la prueba más sensible para diagnosticar enfermedad tromboembólica y CID en medicina humana, y también ha demostrado ser un buen predictor de mortalidad. En medicina equina, elevaciones plasmáticas de dímero-D se han asociado con hipercoagulabilidad en caballos con cólico y septicemia en potros, y algunos estudios defienden su utilidad para ayudar a formular un pronóstico en caballos con problemas gastrointestinales agudos.

Los objetivos de esta tesis doctoral han sido profundizar en el estudio de las coagulopatías en caballos con cólico para mejorar la detección precoz y el tratamiento de la coagulación intravascular diseminada, así como evaluar las posibles aplicaciones clínicas de la medición de la concentración plasmática de dímero-D en caballos con cólico.

Con tal fin se llevaron a cabo tres estudios en una población hospitalaria de caballos con diferentes diagnósticos de enfermedad gastrointestinal, en la que se evaluó el perfil hemostático (incluyendo dímero-D, tiempo de protrombina, tiempo de tromboplastina parcial activada y antitrombina III) y la presencia de CID laboratorial en tres momentos diferentes de la enfermedad: el ingreso,

durante la hospitalización y antes de morir. Además, en el tercer estudio, estos datos clínico-patológicos fueron comparados con datos histopatológicos de los mismos caballos que evaluaban la presencia y cantidad de depósitos microvasculares de fibrina con el fin de detectar cualquier correlación.

Los resultados del primer estudio sugieren que la medición de la concentración plasmática de dímero-D en el momento de la admisión puede contribuir al diagnóstico ya la emisión de un pronóstico en caballos con cólico. Por otra parte, este estudio aporta información novedosa y de utilidad clínica, proponiendo varios puntos de corte para uso pronóstico y diagnóstico. Durante la hospitalización (segundo estudio), la evaluación de la concentración plasmática de dímero-D ha demostrado ser una herramienta útil para monitorizar estados de hipercoagulación en casos de cólico equino. Así mismo, y confirmando resultados de estudios previos, se observaron elevaciones importantes en la concentración de dímero-D en la fase postoperatoria. Por último, el tercer estudio concluyó que la concentración plasmática de dímero-D previa a la muerte no es más útil que el resto de parámetros hemostáticos evaluados para predecir qué pacientes presentan microtrombos de fibrina compatibles con CID en la necropsia.

En conjunto, los tres estudios en esta tesis contribuyen a un mejor conocimiento de las coagulopatías en caballos con problemas gastrointestinales y han proporcionado información novedosa acerca de las posibles aplicaciones de la determinación plasmática de dímero-D en diferentes etapas del síndrome cólico.

Resum

La coagulació intravascular disseminada (CID) és una complicació freqüent en cavalls amb problemes gastrointestinals (GI) greus que ha estat associada amb un mal pronòstic. La CID té com a conseqüència el dipòsit microvascular de fibrina que pot acabar causant disfunció multiorgànica . Estudis experimentals recents han demostrat que la detecció i tractament precoç de les coagulopaties subclíniques permet reduir la morbiditat i mortalitat associades amb la CID. La concentració plasmàtica de dímer-D es considera la prova més sensible per diagnosticar pacients amb malaltia tromboembòlica i CID en medicina humana, i també ha demostrat ser un bon predictor de mortalitat. En medicina equina, elevacions plasmàtiques de dímer-D s'han associat amb hipercoagulabilitat en cavalls amb còlic i septicèmia en poltres, i alguns estudis defensen la seva utilitat per ajudar a formular un pronòstic en cavalls amb problemes gastrointestinals aguts.

Els objectius d'aquesta tesi doctoral han estat aprofundir en l'estudi de les coagulopaties en cavalls amb còlic per millorar la detecció precoç i el tractament de la coagulació intravascular disseminada, així com avaluar les possibles aplicacions clíniques de mesurar la concentració plasmàtica de dímer-D en cavalls amb còlic .

Per això es van dur a terme tres estudis en una població hospitalària de cavalls amb diferents diagnòstics de malaltia gastrointestinal, en què es va avaluar el perfil hemostàtic (incloent dímer-D, temps de protrombina, temps de tromboplastina parcial activada i antitrombina III) i la presència de CID laboratorial en tres moments diferents de la malaltia: l’ ingrés, durant

l'hospitalització i abans de morir. A més, en el tercer estudi, aquestes dades clínico-patològiques van ser comparades amb dades histopatològiques dels mateixos cavalls que avaluaven la presència i quantitat de dipòsits microvasculars de fibrina per tal de detectar qualsevol correlació .

Els resultats del primer estudi suggereixen que la determinació de la concentració plasmàtica de dímer-D en el moment de l'admissió pot contribuir al diagnòstic i a l'emissió d'un pronòstic en cavalls amb còlic. A més a més, aquest estudi aporta informació nova i d'utilitat clínica , proposant diversos punts de tall amb utilitat pronòstica i diagnòstica. Durant l'hospitalització (segon estudi), l'avaluació de la concentració plasmàtica de dímer-D ha demostrat ser una eina útil per monitoritzar estats d'hipercoagulació en cavalls amb còlic. Així mateix, i confirmant resultats d'estudis previs, es van observar importants elevacions plasmàtiques de dímer-D en la fase postoperatòria. Finalment, el tercer estudi va concloure que la concentració plasmàtica de dímer-D prèvia a la mort no és més útil que la resta de paràmetres hemostàtics avaluats per predir quins pacients presenten microtrombes de fibrina compatibles amb CID a la necròpsia .

En conjunt, els tres estudis d’aquesta tesi contribueixen a un millor coneixement de les coagulopaties en cavalls amb problemes gastrointestinals i han proporcionat nova informació sobre les possibles aplicacions de la determinació plasmàtica de dímer-D en diferents etapes del còlic equí.

References

References

1. **Adam** SS, Key NS, Greenberg CS. D-dimer Antigen: Current Concepts and Future Prospects. Blood 2009;113:2878-2887.
2. **Andreasen** EB, Tranholm M, Wiinberg B, et al. Haemostatic alterations in a group of canine cancer patients are associated with cancer type and disease progression. Acta Vet Scand. 2012;54:3.
3. **Armengou** L, Monreal L, Segura D, et al. Plasma D-dimers in horses with colic. Proceedings of the 8th International Equine Colic Research Symposium; 2005 pp2-5; Quebec, Canada (abstract).
4. **Armengou** L, Monreal L, Tarancón I, et al. Plasma D-dimer concentration in sick newborn foals. J Vet Intern Med 2008;22:411-417.
5. **Bakhtiari** K, Meijers JC, de Jonge E, et al. Prospective validation of the international society of thrombosis and haemostasis scoring system for disseminated intravascular coagulation. Crit Care Med 2004;32:2416-2421.
6. **Bateman** SW, Mathews KA, Abrams-Ogg AC, et al. Diagnosis of disseminated intravascular coagulation in dogs admitted to an intensive care unit. J Am Vet Med Assoc. 1999;215:798-804.
7. **Bauer** N, Mensinger S, Daube G, et al. A moderate aseptic local inflammation does not induce a significant systemic inflammatory response. Res Vet Sci. 2012;93:321-330.
8. **Bick** R. Disseminated intravascular coagulation. Objective criteria for diagnosis and management. Med Clin North Am 1994;78:511–543.
9. **Bick** RL. Disseminated intravascular coagulation: a review of etiology, pathophysiology, diagnosis, and management: guidelines for care. Clin Appl Thromb Hemost. 2002;8:1-31.
10. **Brainard** BM, Epstein K, LoBato D, et al. Effects of Clopidogrel and Aspirin on Platelet Aggregation, Thromboxane Production, and Serotonin Secretion in Horses J Vet Intern Med 2011;25:116–122.

11. **Brianceau** P, Divers TJ. Acute thrombosis of limb arteries in horses with sepsis: five cases (1988-1998). Equine Vet J. 2001;33:105-109.
12. **Brooks** M, Leith GS, Allen AK, et al. Bleeding disorder (von Willebrand disease) in a quarter horse. J Am Vet Med Assoc. 1991;198:114.
13. **Brooks** MB. Equine coagulopathies. Vet Clin North Am Equine Pract 2008;24:335-355.
14. **Byars** TD, Thorpe PE. Prognostic significance of fibrin/fibrinogen degradation products in the surgical equine abdominal crisis. Proceedings of the 1st International Equine Colic Research Symposium; 1982 pp28-30; Athens, Georgia, USA (abstract).
15. **Cesarini** C, Monreal L, Armengou L, et al. Association of admission plasma D-dimer concentration with diagnosis and outcome in horses with colic. J Vet Intern Med. 2010;24:1490-1497.
16. **Cesarini** C, Monreal L, Armengou L, et al. Hemostatic follow up of horses with medical and surgical colic. J Vet Intern Med 2009;23:434 (abstract).
17. **Collatos** C, Barton M, Moore J. Fibrinolytic activity in plasma from horses with gastrointestinal diseases: changes associated with diagnosis, surgery and outcome. J Vet Intern Med 1995;9:18–23 **(a)**
18. **Collatos** C, Barton MH, Prasse KW, et al. Intravascular and peritoneal coagulation and fibrinolysis in horses with acute gastrointestinal tract diseases. J Am Vet Med Assoc 1995;207:465-470. **(b)**
19. **Cotovio** M, Monreal L, Armengou L, et al. Fibrin deposits and organ failure in newborn foals with severe septicemia. J Vet Intern Med. 2008;22:1403-1410.
20. **Cotovio** M, Monreal L, Navarro M, et al. Detection of fibrin deposits in tissues from horses with severe gastrointestinal disorders. J Vet Intern Med 2007;21:308-313. **(a)**
21. **Cotovio** M, Monreal L, Navarro M, et al. Detection of fibrin deposits in horse tissues by immunohistochemistry. J Vet Intern Med. 2007;21:1083-1089. **(b)**

22. **Dallap** BL, Dolente B, Boston R. Coagulation Profiles in 27 Horses with Large Colon Volvulus. J Vet Emerg Crit Care 2003;13:215-225.
23. **Dallap** BL. Coagulopathy in the equine critical care patient. Vet Clin North Am Equine Pract 2004;20:231-251.
24. **Darien** BJ, Potempa J, Moore JN, et al. Antithrombin III activity in horses with colic: an analysis of 46 cases. Equine Vet J. 1991;23:211-214.
25. **de Laforcade** AM, Freeman LM, Shaw SP, et al. Hemostatic changes in dogs with naturally occurring sepsis. J Vet Intern Med. 2003;17:674-679.
26. **Delgado** M, Monreal L, Armengou L, Ríos J, Segura D. Peritoneal D-dimer Concentration for Assessing Peritoneal Fibrinolytic Activity in Horses with Colic. J Vet Intern Med 2009;23:882-889.
27. **Divers** TJ. Prevention and Treatment of Thrombosis, Phlebitis, and Laminitis in Horses with Gastrointestinal Diseases. Vet Clin North Am Equine Pract 2003;19:779-790.
28. **Dolente** B, Wilkins P, Boston R. Clinicopathologic evidence of disseminated intravascular coagulation in horses with acute colitis. J Am Vet Med Assoc 2002;220:1034–1038.
29. **Dolente** BA, Beech J, Lindborg S, Smith G. Evaluation of Risk Factors for Development of Catheter-Associated Jugular Thrombophlebitis in Horses: 50 Cases (1993-1998). J Am Vet Med Assoc 2005;227:1134-1141.
30. **Donahue** SM, Otto CM. Thromboelastography: a tool for measuring hypercoagulability, hypocoagulability, and fibrinolysis. J Vet Emerg Crit Care 2005, 15: 9–16.
31. **Dukti** S, White NA. Prognosticating equine colic. Vet Clin North Am Equine Pract. 2009;25:217-31.
32. **Dunkel** B, Chan D, Boston R, et al. Association between Hypercoagulability and Decreased Survival in Horses with Ischemic or Inflammatory Gastrointestinal Disease. J Vet Intern Med 2010;24:1467-1474.
33. **Edens** LM, Morris DD, Prasse KW, et al. Hypercoagulable state associated with a deficiency of protein C in a thoroughbred colt. J Vet Intern Med 1993;7:190-193.

34. **Estrin** MA, Wehausen CE, Jessen CR, et al. Disseminated intravascular coagulation in cats. J Vet Intern Med. 2006;20:1334-1339.
35. **Feige** K, Kästner S, Dempfle C, Balestra E. Changes in Coagulation and Markers of Fibrinolysis in Horses Undergoing Colic Surgery. J Vet Med A Physiol Pathol Clin Med 2003;50:30-36. **(a)**
36. **Feige** K, Schwarzwald C, Bombeli T. Comparison of Unfractioned and Low Molecular Weight Heparin for Prophylaxis of Coagulopathies in 52 Horses with Colic: A Randomised double-blind Clinical Trial. Equine Vet J 2003;35:506-513. **(b)**
37. **Fry** MM, Walker NJ, Blevins GM, et al. Platelet function defect in a thoroughbred filly. J Vet Intern Med. 2005;19:359-362.
38. **Furr** MO, Lessard P, White NA. Development of a colic severity score for predicting the outcome of equine colic. Vet Surg 1995;24:97-101.
39. **Geor** RJ, Jackson ML, Lewis KD, et al. Prekallikrein deficiency in a family of Belgian horses. J Am Vet Med Assoc. 1990;197: 741-745.
40. **Gómez** C, Páramo JA, Colucci M, Rocha E. Effect of heparin and/or antithrombin III on the generation of endotoxininduced plasminogen activator inhibitor. Thromb Haemost 1989;62:694–698.
41. **Hayashi** K, Hsueh CL, Kawasaki H, et al. Importance of immunoenzyme histochemical reaction in diagnosis of disseminated intravascular coagulation in human and animal material. Acta Med Okayama.1989;43:29-38.
42. **Heath** MF, Evans RJ, Poole AW, et al. The effects of aspirin and paracetamol on the aggregation of equine blood platelets. J Vet Pharmacol Ther 1994;17:374–378.
43. **Hoffman** M, Monroe DM 3rd. A cell-based model of hemostasis. Thromb Haemost. 2001;85:958-965.
44. **Holland** M, Kelly A, Snyder J, et al. Antithrombin III activity in horses with large colon torsion. Am J Vet Res 1986;47:897-900.
45. **Ihler** CF, Venger JL, Skjerve E. Evaluation of clinical and laboratory variables as prognostic indicators in hospitalised gastrointestinal colic horses. Acta Vet Scand 2004;45:109-118.

46. **Johnstone** I, Crane S. Hemostatic Abnormalities in Equine Colic. Am J Vet Res 1986;47:356-358. **(a)**
47. **Johnstone** IB, Crane S. Hemostatic abnormalities in horses with colic – their prognostic value. Equine Vet J 1986;18:271-274. **(b)**
48. **Jones** RS, Payne-Johnson CE, Seymour CJ. Pulmonary micro-embolism following orthopaedic surgery in a Thoroughbred gelding. Equine Vet J. 1988;20:382-384.
49. **Katsumura** Y, Ohtsubo K. Association between pulmonary microthromboembolism and coagulation variables in hypercoagulable states: an autopsy study. Respirology. 1999;4:239-243.
50. **Kaufman** HH, Hui KS, Mattson JC, et al. Clinicopathological correlations of disseminated intravascular coagulation in patients with head injury. Neurosurgery 1984;15:34–42.
51. **Kawasugi** K, Wada H, Hatada T, et al. Prospective evaluation of hemostatic abnormalities in overt DIC due to various underlying diseases. Thromb Res 2011; 128: 186-190.
52. **Kim** HS, Suzuki M, Lie JT, et al. Clinically unsuspected DIC - an autopsy survey. Am J Clin Pathol 1976;66:31-39.
53. **Kirby** R, Rudloff E. Acquired coagulopathy VI: disseminated intravascular coagulation. In: Feldman BF, Zinkl JG, Jain NC, editors. Schalm's veterinary hematology. Lippincott, Williams & Williams; 2000. p. 581-587.
54. **Kojima** M, Shimamura K, Mori N, et al. A histological study on microthrombi in autopsy cases of DIC. Bibl Haematol 1983;49:95-106.
55. **Lassen** ED, Swardson CJ. Hematology and hemostasis in the horse: normal functions and common abnormalities. Vet Clin North Am Equine Pract 1995;11:351-389.
56. **Machida** T, Kobubu H, Matsuda K, et al. Clinical Use of D-Dimer Measurement for the Diagnosis of Disseminated Intravascular Coagulation in Dogs. J Vet Med Sci 2010;72:1301-1306.
57. **Marshall** J. Inflammation, coagulopathy, and the pathogenesis of multiple organ dysfunction syndrome. Crit Care Med 2001;29 (7 Suppl):S99–104.

58. **Meyers** KM, Lindner C, Grant B. Characterization of the equine platelet aggregation response. Am J Vet Res 1979;40:260–264.
59. **Moldal** ER, Kristensen AT, Peeters ME, et al. Hemostatic response to surgical neutering via ovariectomy and ovariohysterectomy in dogs. Am J Vet Res. 2012;73:1469-1476.
60. **Monreal** L, Anglés A, Espada Y, et al. Hypercoagulation and hypofibrinolysis in horses with colic and DIC. Equine Vet J 2000;32 Suppl:19-25.
61. **Monreal** L, Cesarini C. Coagulopathies in Horses with Colic. Vet Clin North Am Equine Pract 2009;25:247-258.
62. **Monreal** L. D-dimer as a new test for the diagnosis of DIC and thromboembolic disease. J Vet Intern Med 2003;17:757-759.
63. **Monreal** L. Disorders of the Hematopoietic System. In: Corley K, Stephen J, editors. Equine Internal Medicine. Blackwell Publishing; 2008. p. 401-416.
64. **Montes** R, Rodríguez-Whilhelmi P, Hurtado V, et al. The endotoxin-induced plasminogen activator inhibitor-1 increase in rabbits is not tumor necrosis factor-alpha dependent and can occur in the absence of interleukin-1beta. Thromb Haemost 2002;88: 639–643.
65. **Moore** R, Couto C, Muir W, Moore B, Kociba G. Systemic and Colonic Venous Hemostatic Alterations in Horses during Low-Flow Ischemia and Reperfusion of the Large Colon. Am J Vet Res 1995;56:664-670.
66. **Morris** D. Thrombophlebitis in Horses: The Contribution of Hemostatic Dysfunction to Pathogenesis. Compend Contin Educ Vet. 1989;11.
67. **Morris** DD, Vaala WE, Sartin E. Protein-losing enteropathy in a yearling filly with subclinical disseminated intravascular coagulation and autoimmnune hemolytic disease. Compend Cont Educ Pract Vet 1982;4:542-546.
68. **Nelson** OL, Andreasen C. The Utility of Plasma D-dimer to Identify Thromboembolic Disease in Dogs. J Vet Intern Med 2003;17:830-834.
69. **Norman** T, Chaffin M, Perris E, et al. Massive Pulmonary Thromboembolism in Six Horses. Equine Vet J 2008;40:514-517.

70. **Orsini** JA, Elser AH, Galligan DT, et al. Prognostic index for acute abdominal crisis (colic) in horses. Am J Vet Res 1988;49:1969-1971.
71. **Pablo** LS, Purohit RC, Teer PA, et al. Disseminated intravascular coagulation in experimental intestinal strangulation obstruction in ponies. Am J Vet Res. 1983;44:2115-2122.
72. **Parry** BW, Anderson GA, Gay CC. Prognosis in equine colic: a study of individual variables used in case assessment. Equine Vet J 1983;15:337-344.
73. **Pascoe** PF, Ducharme NG, Ducharme GR, et al. A computer-derived protocol using recursive partitioning to aid in estimating prognosis of horses with abdominal pain in referral hospitals. Can J Vet Res 1990;54:373-378.
74. **Pelagalli** A, Lombardi P, d'Angelo D, et al. Species variability in platelet aggregation response to different agonists. J Comp Pathol 2002;127:126–132.
75. **Prasse** KW, Topper MJ, Moore JN, et al. Analysis of hemostasis in horses with colic. J Am Vet Med Assoc. 1993;203:685-693.
76. **Puotunen-Reinert** A. Study of variables commonly used in examination of equine colic cases to assess prognostic value. Equine Vet J 1986;18:275-277.
77. **Reeves** MJ, Curtis CR, Salman MD, et al. Prognosis in equine colic patients using multivariable analysis. Can J Vet Res 1989;53:87-94.
78. **Robboy** SJ, Colman RW, Minna JD. Pathology of disseminated intravascular coagulation (DIC) - analysis of 26 cases. Hum Pathol 1972;3:327-343.
79. **Roberts** HR, Hoffman M, Monroe DM. A cell-based model of thrombin generation. Semin Thromb Hemost. 2006;32 Suppl 1:32-38.
80. **Rodriquez-Erdman** F. Bleeding due to increased intravascular blood coagulation: hemorrhagic syndromes caused by consumption of blood-clotting factors (consumption coagulopathies). N Engl J Med 1965;273:1370 (abstract).
81. **Rosser** EJ. Use of the D-dimer assay for diagnosing thrombosis in cases of canine cutaneous vasculitis. Vet Dermatol. 2009;20:586-590.

82. **Sandholm** M, Vidovic A, Puotunen-Reinert A, et al. D-dimer improves the prognostic value of combined clinical and laboratory data in equine gastrointestinal colic. Acta Vet Scand 1995;36:255-272.
83. **Scott-Moncrieff** JC, Treadwell NG, McCullough SM, et al. Hemostatic abnormalities in dogs with primary immune-mediated hemolytic anemia. J Am Anim Hosp Assoc. 2001;37:220-227.
84. **Segura** D, Monreal L, Espada Y, et al. Assessment of a platelet function analyser in horses: Reference range and influence of a platelet aggregation inhibitor. Vet J 2005;170:108–112.
85. **Sellon** DC, Wise LN. Disorders of the Hematopoietic System. In: Reed SM, Bayly WM, Sellon DC, editors. Equine Internal Medicine. Saunders; 2010. p. 158-169.
86. **Smith** SA. Update on diagnosis of disseminated intravascular coagulation. Proceedings of the 23th ACVIM Annual Forum; 2005; Baltimore, USA.
87. **Sobiech** P, Targoński R, Stopyra A, et al. Changes in the blood coagulation profile after ovariohysterectomy in female dogs. Pol J Vet Sci. 2011;14:289-290.
88. **Southwood** LL, Dolente BA, Lindborg S, et al. Short-term outcome of equine emergency admissions at a university referral hospital. Equine Vet J 2009;41:459-464.
89. **Stokol** T, Brooks M, Erb H, et al. Evaluation of kits for the detection of fibrin(ogen) degradation products in dogs. J Vet Intern Med. 1999;13:478-484.
90. **Stokol** T, Brooks MB, Erb HN, et al. D-dimer concentrations in healthy dogs and dogs with disseminated intravascular coagulation. Am J Vet Res. 2000;61:393-398. Erratum in: Am J Vet Res 2000;61:728.
91. **Stokol** T, Erb HN, De Wilde L, et al. Evaluation of latex agglutination kits for detection of fibrin(ogen) degradation products and D-dimer in healthy horses and horses with severe colic. Vet Clin Pathol 2005;34:375-382.

92. **Stokol** T. Disseminated intravascular coagulation. In: Weiss DJ, Wardrop KJ, editors. Schalm’s veterinary hematology. Wiley-Blackwell; 2010. p. 679-688.
93. **Stokol** T. Plasma D-dimer for the diagnosis of thromboembolic disorders in dogs. Vet Clin North Am Small Anim Pract. 2003;33:1419-1435.
94. **Tanaka** K, Imamura T. Incidence and clinicopathological significance of DIC in autopsy cases. Bibl Haematol 1983;49:73-93.
95. **Taylor** F. The inflammatory–coagulant axis in the host response to gram-negative sepsis: regulatory roles of proteins and inhibitors of tissue factor. New Horiz 1994;2:555–565.
96. **Taylor** FB, Toh CH, Hoots WK, et al. Towards definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation. Thromb Haemost. 2001;86:1327-30.
97. **Thomas** JS, Green RA. Clotting times and antithrombin III activity in cats with naturally developing diseases: 85 cases (1984-1994). J Am Vet Med Assoc. 1998;213:1290-1295.
98. **Toh** CH, Downey C. Back to the future: testing in disseminated intravascular coagulation. Blood Coagul Fibrinolysis. 2005;16:535-542.
99. **Topper** MJ, Prasse KW. Analysis of coagulation proteins as acute-phase reactants in horses with colic. Am J Vet Res. 1998;59:542-545.
100. **Topper** MJ, Prasse KW. Use of enzyme-linked immunosorbent assay to measure thrombin-antithrombin III complexes in horses with colic. Am J Vet Res. 1996;57:456-462.
101. **Traub-Dargatz** J, Dargatz D. A Retrospective Study of Vein Thrombosis in Horses Treated with Intravenous Fluids in a Veterinary Teaching Hospital. J Vet Intern Med 1994;8:264-266.
102. **Triplett** EA, O'Brien RT, Wilson DG, et al. Thrombosis of the brachial artery in a foal. J Vet Intern Med. 1996;10:330-332.
103. **Tripodi** A. D-dimer Testing in Laboratory Practice. Clin Chem 2011;57:1256-1262.

104. **Wada** H, Sakuragawa N, Mori Y, et al. Hemostatic molecular markers before the onset of disseminated intravascular coagulation. Am J Hematol 1999;60:273-278.

105. **Wada** H, Thachil J, Di Nisio M, et al. Guidance for diagnosis and treatment of disseminated intravascular coagulation from harmonization of the recommendations from three guidelines. J Thromb Haemost 2013;11:761–767.

106. **Wada** H, Wakita Y, Nakase T, et al. Outcome of disseminated intravascular coagulation in relation to the score when treatment was begun. Thromb Haemost 1995;74:848-852.

107. **Watts** AE, Fubini SL, Todhunter RJ, et al. Comparison of plasma and peritoneal indices of fibrinolysis between foals and adult horses with and without colic. Am J Vet Res. 2011;72:1535-1540.

108. **Welch** R, Watkins J, Taylor T, Cohen N, Carter G. Disseminated intravascular coagulation associated with colic in 23 horses (1984–1989). J Vet Intern Med 1992;6:29–35.

109. **Welles** E, Prasse K, Moore J. Use of Newly Developed Assays for Protein C and Plasminogen in Horses with Signs of Colic. Am J Vet Res 1991;52:345.

110. **Wiinberg** B, Jensen AL, Johansson PI, et al. Development of a model based scoring system for diagnosis of canine disseminated intravascular coagulation with independent assessment of sensitivity and specificity. Vet J. 2010;185:292-298.

111. **Wiinberg** B, Jensen AL, Johansson PI, et al. Thromboelastographic evaluation of hemostatic function in dogs with disseminated intravascular coagulation. J Vet Intern Med. 2008;22:357-365.

112. **Wiinberg** B, Jensen AL, Rojkjaer R, et al. Prospective pilot study on performance and prognostic value of a new human, ISTH-based scoring ystem for identifying non-overt disseminated intravascular coagulation in dogs. J Vet Intern Med 2006;20:765 (abstract).

113. **Wilde** JT, Roberts KM, Greaves M, et al. Association between necropsy evidence of disseminated intravascular coagulation and coagulation variables before death in patients in intensive care units. J Clin Pathol. 1988;41:138-142.

114. **Wilson** I, Gamble M. The hematoxylins and eosin. In:Bancroft JD, Gamble M, eds. Theory and Practice of Histological Techniques. Philadelphia, PA: Churchill Livingstone; 2002:125–138.

Notes